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Genetics

Principal Investigator:
Grant Number: N01NS022349
Title: Genetic Resource Center

Abstract: Unavailable

Principal Investigator: ABELIOVICH, ASA
Grant Number: 5R01NS046659-02
Title: Molecular and Cellular Analyses of Parkin Function

Abstract: Defective protein degradation through the ubiquitin proteasome pathway (UPP) has been hypothesized to play a central role in neurodegenerative disorders such as Parkinson's disease (PD). Mutations in Parkin, a putative ubiquitin ligase component, cause a familial, autosomal recessive form of PD characterized by midbrain dopamine neuron loss. It has therefore been hypothesized that inefficient degradation and consequent toxic accumulation of Parkin ubiquitination substrates underlie the loss of dopamine neurons in autosomal recessive Parkinson's disease. We further hypothesize that Parkin may play a direct role in regulating neuronal survival in the CNS. We propose to use molecular and cellular tools to investigate the mechanism of Parkin action in protein ubiquitination and neuronal survival. Our preliminary data indicate that Parkin associates in a multiprotein ubiquitin ligase complex with 2 previously characterized ubiquitin ligase components, the F-box/WD repeat-containing protein hSel- 10, and Cullin-1 (Cul 1). Furthermore, hSel-10 serves to direct this complex to specific substrates including Cyclin E, a putative regulator of neuronal apoptosis. We will test the hypotheses that (1) auxiliary components of the Parkin ubiquitin ligase complex serve to regulate or target this activity, and (2) that, in Parkin-associated familial PD, premature neuronal death is a consequence of defective ubiquitination and the accumulation of neuronal apoptosis-related Parkin complex substrates. -

Principal Investigator: ALBUQUERQUE, EDSON
Grant Number: 5R01NS041671-04
Title: Nicotinic Receptors in Septally Innervated Hippocampus

Abstract: The dysfunction and degeneration of the nicotinic cholinergic system in the brain are integral physiopathological indicators of one of the most socially impacting neurological disorders, Alzheimer's disease (AD). In AD, the permanent loss of cholinergic neurons and nicotinic receptors (nAChRs) in brain areas that process cognitive functions, particularly the hippocampus and the frontal cortex, correlates well with the decline in cognition and memory. To date, treatment of patients with AD relies heavily on the use of acetylcholinesterases. These drugs, by increasing function of the cholinergic system, partially reverse the symptoms of AD patients. Recently, clinical trials have shown that nicotinic agonists (including nicotine) and drugs that allosterically potentiate the activity of nAChRs are more effective for treatment of patients with AD. The mechanisms underlying the effectiveness of these drugs remain unknown, because there is very little information on how function and expression of neuronal nAChRs in the brain are regulated by cholinergic afferents. In addition, detailed analysis of regulation of nAChR expression and function in the hippocampus by septal cholinergic afferents has been limited by the lack of a viable biological preparation that closely resembles the nicotinic cholinoceptive hippocampal system in vivo. Our initial characterization of the nicotinic properties of hippocampal neurons in organotypic, hippocampal and septal-hippocampal cultures constitutes the mainstay of the present proposal, as it establishes the septal-hippocampal co-cultures as an excellent model for in vitro study of the influences of septal innervation on nAChR expression in the hippocampus. Thus, this proposal is designed to use convergent, multidisciplinary approaches to address the central hypothesis that septal innervation and nicotine dynamically modify the hippocampal cholinergic system. The first goal of this study is to use electrophysiology, confocal microscopy, ligand binding and immunocytochemistry to determine whether septal innervation alters the nicotinic properties of different types of hippocampal neurons during development in organotypic cultures. The second goal is to use electrophysiological assays, recombinant DNA technology and "knock-out" mice, which have a null mutation in the gene encoding alpha7 nicotinic receptors, to study nAChR targeting and to investigate the motifs in the nAChR subunits that account for final receptor targeting in hippocampal neurons. The final goal is to use electrophysiological, biochemical and molecular biological techniques to evaluate how nicotine affects alpha7 and alpha4beta2 nAChR expression in the hippocampus. The results of these studies will have far

Principal Investigator: ARNOLD, ARTHUR P
Grant Number: 5R01NS045966-02
Title: Sex Differences in Dopamine Systems

Abstract: The proposal has the long term goal of determining the factors that cause sex differences in structure, function, and susceptibility to disease in mesencephalic dopamine systems. The studies will investigate the cellular and molecular mechanisms by which sex chromosome genes induce sex differences in phenotype of dopaminergic neurons in vivo and in vitro. Studies will determine whether the sex chromosome effect is due to genes on the X or Y chromosomes; whether steroid hormones of the Sry gene participate in the induction of sex differences; when during development the sex chromosome effect occurs; whether the sex chromosome effect is direct or indirect on dopamine neurons; the cellular mechanisms of the sex chromosome effect; and whether the sex chromosomes contribute to sex differences in the development and adult structure of the nigrostriatal dopamine system in vivo. The proposed studies will contribute to an understanding of the principles of sexual differentiation of the brain. At issue are the molecular mechanisms by which male and female brains differ, which is relevant to the biological basis of abnormalities of sexual differentiation, and to the explanation of sex differences in neurological and psychiatric disease, not only of those that affect dopamine systems (e.g., Parkinson's Disease, Tardive Dyskinesia, Tourette's Syndrome, schizophrenia), but other sexually dimorphic diseases as well. (e.g., Multiple Sclerosis). Understanding sex differences in brain function will help develop sex-specific strategies for treatment of brain diseases.-

Principal Investigator: Aurelian, Laure
Grant Number: 5R01NS045169-02
Title: Neuroprotection and ERK activation by HSV-2 gene ICP10PK

Abstract: Unavailable

Principal Investigator: BONINI, NANCY M
Grant Number: 5R01NS043578-02
Title: Molecular Genetic Analysis of Neurodegeneration

Abstract: Many human neurodegenerative diseases are poorly understood as well as untreatable, including Parkinson's, Alzheimer's and Huntington's diseases. For some familial forms of these diseases, mutations in specific genes products associated with disease are known, allowing the possibility to model the disease in simple systems in order to address mechanisms of degeneration and to pioneer novel treatments. Toward this end, we applied a new approach to the problem of polyglutamine-induced neurodegeneration by developing a model for this class of human disease in the fruit fly Drosophila melanogaster. These experiments demonstrated that fundamental molecular mechanisms of polyglutamine-induced neurodegeneration are conserved in Drosophila, such that Drosophila genetics can be applied to investigate these human diseases in order to address mechanisms of degeneration and define new means of treatment. Using this model, we have shown that the molecular chaperones, which are highly conserved proteins, are potent modulators of neurodegeneration in vivo. We now propose to apply the powerful molecular genetics of Drosophila in genetic screens to uncover additional modulators of neurodegeneration. The advantage of genetic screens is that they provide the ability to define genes that can influence and modulate pathogenesis without requiring previous knowledge of the mechanisms involved. The specific aims are to define novel modulators of neurodegeneration in mis-expression and loss-of-function genetic screens, and to molecularly define and biologically characterize these modifiers in order to address their molecular and biological modes of action. By applying the power of Drosophila molecular genetics to address conserved features of polyglutamine-induced degeneration, these studies provide the foundation for new approaches to cures and treatments for human neurodegenerative disease. -

Principal Investigator: BORCHELT, DAVID R
Grant Number: 1R01NS044278-01A2
Title: Protein Misfolding in Neurodegeneration

Abstract: The accumulation of ubiquitin-immuno-reactive material in cell bodies, dendrites, and/or axons of neurons are a prevalent pathology of neurodegenerative disease. It has been suggested that the accumulation of this material is a cellular symptom of reduced ubiquitin/proteasome system (UPS) function. In the present application, we propose four Aims that are designed to probe whether and how the proteasome/ubiquitin system is dysfunctional in various models of neurodegenerative disease. In Aim 1, we propose to use genetic approaches to alter the activities of UPS components. We have been provided mice lacking parkin (a biquitin E3 ligase whose loss triggers Parkinson's disease), and we would like to cross these mice to our APPswe/PS1dE9 mice. We hypothesize that amyloid deposition, in a context of parkin deficiency, may induce novel cytoplasmic pathologies, such as Lewy-body-like inclusions. Aim 2 will develop systems to inhibit proteasome function in vivo in transgenic mice, both chronically and acutely, using genetic approaches. Aim 3 will build on recent characterization of a subset of sporadic ALS cases, where we have identified cystatin C as a protein of interest in the disease. To test the role of this protein in ALS, we propose to create transgenic animals that express elevated levels of the human protein. Aim 4 will focus on identifying the protein backbone constituents of the ubiquitin immunoreactive material that accumulates in our mouse models of Alzheimer's disease and ALS. This Aim will involve the development of transgenic mice expressing recombinant ubiquitin molecules carrying peptide motifs that facilitate detection and purification. Collectively, these studies should allow us to examine the role of proteasome dysfunction in disease pathogenesis and perhaps identify some of the mis-folded proteins that accumulate in disease-associated inclusions. -

Principal Investigator: Botas, Juan
Grant Number: 5R01NS042179-04
Title: Neurodegeneration with Drosophila

Abstract: The ultimate goal of this project is to gain insight into polyglutamine-induced neurodegeneration by identifying genes, pathways and molecular mechanisms involved in the pathogenesis of spinocerebellar ataxia type 1 (SCA1). A Drosophila model of SCA1 was created by generating flies that express either normal or expanded human SCA1 transgenes. This fly model recapitulates the cellular phenotypes observed in SCA1 patients including the formation of nuclear inclusions (NI) and progressive neuronal degeneration. Capitalizing on the power of Drosophila genetics, two large genetic screens were designed to identify genes that modify a SCA1 neurodegenerative phenotype in the eye. The first screen yielded modifiers of the SCA1 phenotype when gene activity was decreased; the second screen yielded SCA1 modifiers when gene activity was increased. Both suppressors and enhancers of the neurodegenerative phenotype were obtained from each screen. The first aim of the proposed work is to identify the genes that modify the SCA1 neurodegenerative phenotype. These modifiers will be further characterized in sensitive viability and locomoter assays that allow the quantification of their modifier effects. The most powerful suppressors will be selected for further studies. To investigate whether different polyglutamine disease share common mechanisms of pathogenesis, the SCA1 modifiers will be tested in fly models of Huntington disease and polyglutamine toxicity. Finally, because the normal function of the SCA1 gene may be relevant to pathogenesis, the function of the Drosophila SCA1 gene will be investigated by generating lack-of-function mutations and transgenes for its over expression. In future studies, the most promising SCA1 suppressors characterized in flies will be investigated in the SCA1 mouse model, and in mouse models of polyglutamine disease. These genes may also be relevant to research aimed at treating other neurodegenerative proteinopathies such as Alzheimer disease and Parkinson disease. They will provide valuable targets for future pharmacological research aimed at developing drugs for therapy. -

Principal Investigator: BREAKEFIELD, XANDRA
Grant Number: 5R01NS028384-14
Title: Characterization of Dystonia Gene and Protein

Abstract: The long term goal of this research is to understand the function of the novel protein, torsinA, which is mutated in early onset torsion dysconia. This neurologic disease is inherited as an autosomal dominant condition with reduced penetrance and a developmental window of susceptibility in childhood and adolescence. The torsins are members of the AAA+ superfamily of chaperone proteins involved in protein configurational changes. Studies to date suggest that torsinA may be involved in vectorial membrane movement and/or response of cells to oxidative stress. Only two in-frame mutations resulting in loss of amino acids in the carboxy terminal of this protein have been found in patients with early onset dystonia. In these studies we will screen for additional mutations in patients at the genomic and transcript levels. This analysis will be complemented by generation and expression of targeted mutations in the protein to elucidate structure/function determinants of ATPase activity, oligomerization, and posttranslational modifications. Cellular correlates will include the formation of whorled membrane inclusions in cells overexpressing the GAG-deleted form of torsinA found in most patients. In parallel, a search will be undertaken to identify partner proteins and their binding domains to torsinA, using the yeast two hybrid system, co-immunoprecipitation, affinity binding to purified protein, and protein chip assays. Immunocytochemistry will be used to visualize the cellular location of partner proteins relative to torsins.The predicted function of torsinA as a sensor to oxidative Stress will be examined by characterizing the posttranslational modifications to torsins which result from exposure to hydrogen peroxide and by evaluating whether expression of wild type or mutant forms of torsin act to protect or sensitize cells to this oxidative stress. TorsinA's predicted function in membrane and protein movement will be assessed by monitoring the morphology of the endoplasmic reticulum and vesicles, as well as constitutive and regulated secretion of proteins and vesicle recycling in cultured cells and neurons expressing mutant forms. These studies should help elucidate the function of this novel class of chaperone proteins and reveal how specific mutations in torsinA can disrupt cell function. Dystonia represents a special class of neurologic diseases, which do not manifest apparent neurodegeneration. This class of diseases may be amenable to therapy informed by the molecular etiology of dysfunction at the cellular level. -

Principal Investigator: BRICE, ALEXIS
Grant Number: 5R01NS041723-03
Title: Parkin mutations and their functional consequences

Abstract: Parkin, a gene with marked allelic heterogeneity including both point mutations and exon rearrangements, accounts for about 50 percent of early onset (< 45 yrs) autosomal recessive cases of Parkinson's disease in Europe. However, the frequency and types of mutations, particularly in late onset cases, their consequences on Parkin expression and the clinical features of the disease are not well known. We will recruit a unique series of PD cases, isolated or familial (autosomal dominant or recessive), with early or late onset, evaluated with standard criteria by a network of movement disorders specialists in Europe, Mediterranean countries, Brazil and Russia, to exhaustively determine the spectrum of parkin mutations and their origin (de novo, founder effects). All patients will be screened by semi quantitative multiplex PCR and DHPLC for mutations in parkin coding sequences. In selected patients with possibly undetected or dominant mutations, coding and promoter regions will be analyzed by combinations of direct sequencing, RT-PCR, Southern blot, FISH to detect mutations affecting regulatory regions or unusual mutational mechanisms (inversions). The consequences of the mutations on mRNA (RT-PCR) and protein (Western blot) expression will pennit correlations between genotypes and phenotypes in relation to their effects on Parkin function and in comparison with other forms of PD. In addition, an association study of 4 polymorphisms in the parkin coding sequence in 400 patients and matched controls will determine whether parkin is a risk factor for other forms of PD. Finally, families excluded from the parkin and the PARK6 loci will be used in a genome-wide search for other loci/genes responsible for early onset autosomal recessive PD. This comprehensive study of the parkin gene will contribute important information both for basic research (consequences of mutations, parkin as a risk factor) and clinical practice (phenotype(s), molecular diagnosis) and enlarge the spectrum of loci/genes responsible for monogenic forms of PD. -

Principal Investigator: BROADIE, KENDAL S
Grant Number: 5R01NS041740-05
Title: Synaptic Mechnisms in Drosophila Neurodegeneration Model

Abstract: The hypothesis driving this proposal is that presynaptic dysfunction is a common causative factor leading to cell death in multiple inherited neurodegenerative diseases. This hypothesis is based on the observations that 1) synaptic function mediates neuronal survival during development, 2) mutations which strongly impair presynaptic function result in massive, progressive neuronal degeneration, 3) a number of presynaptic proteins have been directly implicated in neurodegenerative diseases and 4) neuronal dysfunction/synapse loss is known to precede by a substantial period the manifestation of cell death in these diseases. To date, however, there is no established direct evidence of synaptic dysfunction mediating neuronal death during neurodegenerative disease states. The goal of this proposal is to assay synaptic maintenance in two genetic models of neurodegenerative diseases: Drosophila models of Parkinson's Disease (PD), a classic "protein storage" disease, and Niemann-Pick Type C (NP-C), a classic "lipid storage" disease. Drosophila was selected for its attractive properties as a new molecular genetic model of neurodegeneration, and its long history as the foremost genetic model for synaptic studies. PD and NP-C were selected as representative of a large number of related neurodegenerative disorders. The Drosophila PD model has been recently established through transgenic over-expression of human alpha-synuclein (a presynaptic protein) and shown to accurately recapitulate the diagnostic features of human PD. A Drosophila model of NP-C is being established through mutation (loss-of-function) of the endogenous NPC I gene, the known cause of human NP-C disease. Specifically, this proposal is to conduct age-progressive studies of synaptic mechanisms in Drosophila PD and NP-C models to correlate synaptic maintenance with the onset, progression and prevalence of neurodegeneration. The first aim is to improve Drosophila models by generating fluorescently tagged alpha-synuclein and NPCI proteins whose levels can be reversibly regulated through a temperature-dependent ubiquination strategy. Secondly, to confirm gross features of neurodegeneration in these models with behavioral assays and examination of nervous system/neuronal architecture. Third, and most importantly, to assay synaptic development, function and maintenance in these models. Assays will include electrophysiological measurements of neurotransmission, quantitative fluorescent optical imaging of protein and lipid dynamics in the presynaptic terminal and ultrastructural studies of presynaptic architecture. Together, these studies will allow a conclusive determination of whether synaptic maintenance is

Principal Investigator: BURKE, ROBERT E
Grant Number: 2P50NS038370-06
Title: Mechanisms of dopamine neuron degeneration

Abstract: Parkinson's disease (PD) is a prevalent and disabling neurological disease characterized by the progressive loss of motor control due to the degeneration of dopamine (DA) neurons of the substantia nigra. Among neurodegenerative diseases, PD has served as a model for the development of novel therapeutic approaches: administration of neurotransmitter precursors (levodopa), cell implantation, and more recently, deep brain stimulation. As important and effective as these advances have been, they only relieve symptoms; none stop the progression of the disease. In order to develop therapies which halt the progression of the disease, we need to achieve a better understanding of the pathogenesis of DA neuron degeneration. This submission represents a competing continuation application for a Morris K. Udall Parkinson's Disease Research Center of Excellence awarded to Columbia University in 1999. This renewal consists of four projects devoted to a single integrating theme: to understand the molecular and cellular mechanisms of dopamine neuron degeneration. While there are many worthy hypotheses of pathogenesis, the subprojects of this proposal will focus on four major current themes in the pathogenesis of PD, related to the roles of: (1) Abnormal intracellular protein degradation; (2) Inflammatory pathways; (3) Programmed cell death (PCD); and (4) Oxidative injury. In Project 1, Dr Serge Przedborski will evaluate the role of cyclooxygenase 2 (COX2) and cytosolic phospholipase A2 (cPLA2) (Theme 2) in mediating dopamine neuron damage in the MPTP model of PD and in human brain samples. In Project 2, Dr David Sulzer will examine in astrocyte and neuron primary cultures the role of chaperone mediated autophagy in the degradation of proteins implicated in PD (Theme 1) and the effect of these proteins on catecholamine sequestration (Theme 4). In Project 3, Dr Robert Burke will use genetic techniques in animal models to examine the roles of the mixed lineage kinases, Akt and JNK in mediating PCD in dopamine neurons (Theme 3), and he will evaluate the functional role of ER stress in initiating cell death (Theme 1). In Project 4, Dr Lloyd Greene will continue to evaluate the functional role of genes identified in the current funding period by SAGE analysis as upregulated following neurotoxin exposure. He will continue his studies of the role of ER stress-related genes (Theme 1) and genes implicated in PCD (Theme 3) in PC12 cells and primary sympathetic neurons, and in living animal models (the latter in collaboration with Drs Burke and Przedborski). He will also examine these transcripts and their protein products in PD brain. -

Principal Investigator: CHEN, HONGLEI
Grant Number: 1K08NS048468-01
Title: Diet, gene-diet interactions and risk of Parkinson's

Abstract: The candidate, Honglei Chen, M.D., Ph.D., has more than two years research experience in Parkinson's disease (PD) and is currently a Research Associate at Harvard School of Public Health. Dr. Chen's research interest includes the environmental and genetic etiology of sporadic PD and that of other neurodegenerative diseases, and he plans to develop an independent academic career in this area. In this K08 proposal, Dr. Chen proposes a large prospective investigation of diet and risk of sporadic PD in the Cancer Prevention Study-ll Nutrition Cohort (CPS-IIn) and a large nested case-control study of PD with genetic polymorphisms and gene-diet interactions in the Health Professionals Follow-up Study (HPFS) and the Nurses' Health Study (NHS). In the CPS-IIn, he will prospectively examine among 162,408 US men and women associations of PD with dietary intakes, focusing on folate, coffee, dietary antioxidants, fat, alcohol, and dairy products. Confirmation of incident PD cases in CPS-IIn is ongoing and they expect to document 550 definite and probable PD cases diagnosed between 1992 and 2001. In the HPFS and NHS cohorts, he will evaluate the associations of PD risk with common polymorphisms of NAT2, CYP1A2, ADH2, ADH3, ADH4, and MTHFR. He also will, for the first time, explore gene-diet interactions in PD etiology, including NAT2, CYP1A2 and caffeine intake; ADH2, ADH3, ADH4, and alcohol intake; and MTHFR and folate intake. Through the year of 2000, they have documented 567 definite and probable PD cases and 454 of them provided either blood or cheek cells for genetic analysis. In this proposed nested case-control study, two controls will be selected for each PD case matching on age and gender. All three cohorts included in this proposal are well-established large prospective cohorts with comprehensive (baseline and updated) and validated dietary assessments and rigorous outcome ascertainments. Moreover, the scope of this study makes it one of the largest investigations to date. The completed or nearly completed data collection will further make this study most cost-effective. Therefore, this K08 grant will simultaneously accomplish two important goals: helping Dr. Chen develop the skills to become an independent researcher in the epidemiology of neurological diseases and furthering our understanding of the complex interrelationships among diet, genes and PD etiology. -

Principal Investigator: DAUER, WILLIAM T
Grant Number: 1K02NS045798-01A1
Title: The mechanism of MPTP resistance in synuclein null mice.

Abstract: My long-held career goal is to investigate questions of importance to both patient care and fundamental biology. During medical training, I developed a strong interest the basic pathogenic mechanisms of Parkinson's disease (PD), an illness characterized by degeneration of substantia nigra dopamine (DA) neurons and cytoplasmic aggregates of alpha-synuclein (SYN). I came to appreciate the power of genetically modified animals as tools to explore basic aspects of disease pathogenesis, and developed expertise in the generation of such animals. However, I now need to acquire skills necessary to assess the consequences of PD-related mutations on cellular and behavioral aspects of dopaminergic function in these animals. To accomplish this goal, I have developed collaborations with experts in PD research, and will pursue the proposed work within the integrated PD research group at Columbia University. Rarely, PD may be caused by missense mutations in SYN. However, normal SYN function and the mechanism by which pathogenic mutations disrupt SYN biology and lead to PD are poorly understood. MPTP-induced degeneration of DA neurons is a commonly studied model of PD. We find that SYN null mice display striking resistance to MPTP-induced degeneration of DA neurons, and this resistance appears to result from an inability of the toxin to access and inhibit its target, mitochondrial complex I. The goal of this research plan is to exploit this robust phenotype of SYN null mice to gain insight into the normal function of SYN, and explore how this function is altered by PD-causing mutations. In Aim 1 we will measure whether known concomitants of complex I inhibition (increased lactate and reactive oxygen species; decreased ATP) are also impaired in SYN null mice, and characterize processes that control access of the toxin to complex I (vesicular and monoamine transporter function). In Aim 2 we will further explore whether altered synaptic function underlies the MTPT resistance of SYN null mice by testing whether they are selectively resistant to toxins that traffic through the synapse. In Aim 3, by restoring wild type or mutant SYN to specific neuronal populations of SYN null mice, we will test whether the MPTP resistance is a cell autonomous phenomenon and whether pathogenic SYN mutations modify an aspect of its function involved in effecting MPTP-induced neurodegeneration. This proposal exemplifies the type of clinically related fundamental neurobiological research I plan to pursue during my career.-

Principal Investigator: DAWSON, TED M
Grant Number: 1R21NS047565-01
Title: Models of Familial Parkinson's Disease: DJ-1 Knockouts

Abstract: Mutations in the DJ-1 gene are a rare genetic cause of autosomal recessive Parkinson's disease (PD). The DJ-1 protein is either absent or appears to be functionally inactive in the families in which mutation have been identified. Thus, mutations in the DJ-1 gene probably cause PD through a loss of function. It is difficult at this juncture to fully appreciate how mutations in the DJ-1 gene cause PD, as its function is largely unknown. DJ-1 was identified as a hydroperoxide-responsive protein that becomes more acidic following oxidative stress suggesting that it may function as an antioxidant protein. Furthermore, DJ-1 is sumoylated through binding to the SUMO-1 ligase, PIAS, suggesting that it might be involved in the regulation of transcription. Other putative functions of DJ-1 have been raised, but how a loss of function of DJ-1 leads to loss of DA neurons and PD awaits further study. We propose to generate and characterize DJ-1 knockout mice to formally test the hypothesis that the absence of DJ-1 function is the cause of PD due to DJ-1 mutations. Accordingly experiments are proposed to further characterize the role of DJ-1 in the pathogenesis of PD. In Specific Aim #1 we will develop and characterize DJ-1 knockout mice. In Specific Aim #2 we will evaluate the sensitivity of DJ-1 knockouts to environmental toxins including MPTP-induced dopaminergic cell death. In Specific Aim #3 we will determine whether DJ-1 interacts with parkin by evaluating the effect of crossing DJ-1 knockout mice with parkin knockout mice. Development and characterization of DJ-1 knockouts, understanding the relationship of DJ-1 and parkin in the pathogenesis of PD may provide insight into the molecular mechanisms by which these gene products induce neuronal damage and may provide novel therapeutics and targets to prevent the toxic effects of these familial associated genes in the degenerative process of PD. -

Principal Investigator: DAWSON, TED M
Grant Number: 2P50NS038377-06A1
Title: Parkinson's Disease Research Center of Excellence

Abstract: The overall goals of this proposal are to understand the role of alpha-synuclein, parkin, DJ-1 and synphilin-1 in the pathogenesis and pathology of Parkinson's disease (PD) and to define the molecular mechanisms of neuronal injury in animal models of PD. The program represents a multi-disciplinary, mechanistic approach involving interactive, productive investigators with complementary areas of expertise who have long been committed to the studies of neurodegenerative diseases. Their aim will be to integrate the activities of various disciplines such that the interrelationships will result in a greater scientific contributions and achievements if each project were pursued individually. The program has one major theme: To understand the role of familial associated genes alpha-synuclein, parkin and DJ-1 in the pathogenesis of Parkinson's disease and related disorders. The role of alpha-synuclein, parkin, DJ-1 and synphilin- 1 in PD pathogenesis will be investigated using molecular, transgenic, neuropathologic, cell biologic and neurobehavioral approaches to examine the mechanism of neuronal dysfunction and injury clue to alterations in these gene products. The mechanism of neuronal loss in Parkin knockout mice and alpha-synuclein A53T transgenic mice will be characterized. We will determine whether parkin interacts with alpha-synuclein and further explore the relation between and parkin, alpha-synuclein and synphilin-1. We will explore alpha-synuclein processing and modifications and the relationship of synphilin-1 to alpha-synuclein. Furthermore, we will investigate the potential function of DJ-1 and it role in PD Pathogenesis. We believe that our multi-disciplinary approach has the capacity to produce unique information concerning the mechanisms of neurodegeneration in genetic animal models of Parkinson's disease and the related synucleinopathies and to lead to better understanding of the function and the role of alpha-synuclein, parkin, DJ-1 and synphilin-1 in normal and pathophysiologic processes related to PD. The program consists of four projects: 1) Mouse Models of Parkin Biology and Pathobiology 2) PD Cell Models: Alpha-synuclein and Interacting Proteins; 3) Mechanisms of Neurodegeneration in Human Alpha-synuclein Transgenic Mice; 4) The Role of DJ-1 in Parkinson's Disease and four cores A) Administration and Training; B) Transgenic and Neurobehavior; C) Neuropathology and D) Clinical.-

Principal Investigator: DAWSON, TED M
Grant Number: 1R01NS048206-01
Title: The Role of Parkin in Parkinson's Disease

Abstract: Mutations in the parkin gene are the main genetic cause of autosomal recessive Parkinson's disease (PD) and mutations in parkin also play a major role in familial Parkinson's disease. Preliminary studies indicate a potential pivotal role for parkin in the ubiquitin proteasomal pathway (UPP) by functioning as an ubiquitin E3 ligase. Most disease causing mutations of parkin are thought to be loss of function mutations that ultimately lead to the absence of ubiquitination and the subsequent failure of UPP-mediated degradation of parkin substrates. Thus, the abnormal accumulation of parkin substrates is thought to play a role in the demise of substantia nigra dopaminergic neurons in patients with parkin mutations. A number of putative parkin substrates have been identified, but their importance in the pathogenesis of PD due to parkin mutations is not known. We propose to generate and characterize parkin knockout mice to formally test the hypothesis that the absence of parkin function is the cause of PD due to parkin mutations. Furthermore, biochemical and proteomic characterization of the parkin knockout mice may shed light on the substrates that are important in the pathogenesis of PD due to parkin mutations. Accordingly experiments are proposed to further characterize the role of parkin and it's substrates in the pathogenesis of Parkinson's disease. In Specific Aim #1 we will develop and characterize parkin knockout mice. In Specific Aim #2 we will evaluate the sensitivity of parkin knockouts to environmental toxins including MPTP-induced dopaminergic cell death. In Specific Aim #3 we will evaluate the interaction of parkin with the alpha-synuclein interacting protein, synphilin-1 and determine whether parkin mediates K48 or K63 ubiquitin linkages. In Specific Aim #4 we will determine whether parkin interacts with alpha-synuclein and evaluate the effect of crossing parkin knockout mice with A53T mutant alpha-synuclein transgenic mice. In Specific Aim #5 we will identify and characterize parkin interacting proteins in parkin knockout mice. Development and characterization of parkin knockout mice, understanding the relationship of parkin, alphasynuclein and synphilin-1 in the pathogenesis of PD may provide insight into the molecular mechanisms by which these gene products induce neuronal damage and may provide novel therapeutics and targets to prevent the toxic effects of this familial associated genes in the degenerative process of Parkinson's disease. -

Principal Investigator: DAWSON, VALINA L.
Grant Number: 5R01NS040809-04
Title: Mechanisms of Ischemic Tolerance

Abstract: The overall goal of this project is to understand the molecular mechanisms of ischemic tolerance in cortical neurons. Neuronal ischemic preconditioning or tolerance is a phenomenon in which brief episodes of ischemia protect against the lethal effects of subsequent periods of prolonged ischemia. The signaling mechanisms leading to preconditioning are poorly understood but have the potential for providing important pharmaceutical targets for the treatment of patients at risk for ischemic injury and possibly the treatment of patients suffering from chronic neurodegenerative diseases such as Parkinson's Disease. Ischemia can be modeled in vitro by oxygen-glucose deprivation (OGD). We have recently discovered that OGD preconditioning induces p2 p21ras (Ras) activation in a NMDA receptor- and NO-dependent manner. OGD preconditioning is dependent on Ras activation of the Raf-Mek-Erk pathway. Our observations indicate that activation of the Ras/Erk cascade by NO is a critical mechanism for the development of OGD tolerance in cortical neurons, which may also play an important role in ischemic preconditioning in vivo. To further our understanding of preconditioning it is essential to identify the transcriptional elements that are activated and the new proteins that are responsible for this remarkable neuroprotection. In this project we propose to investigate the role of transcriptional targets of the Ras/Erk signaling cascade with a focus on CREB and Elk activation. We will identify genes that are regulated by preconditioning and determine which genetic changes are responsible for preconditioning. Preconditioning can also be induced by potassium depolarization in an in vitro model of spreading depression. We will investigate whether similar or different mechanisms are responsible for potassium depolarization induced tolerance. We anticipate that this series of investigations will identify endogenous protective mechanisms that ultimately may be harnessed as novel protective strategies against ischemic and traumatic injury as well as chronic neurodegenerative disorders such as Parkinson's Disease. -

Principal Investigator: DEBBURMAN, SHUBHIK
Grant Number: 3R15NS048508-01S1
Title: Yeast Model for Two Neurodegeneration-Linked Proteins

Abstract: Unavailable

Principal Investigator: DEBBURMAN, SHUBHIK
Grant Number: 1R15NS048508-01
Title: Yeast Model for Two Neurodegeneration-Linked Proteins

Abstract: Budding Yeast (S. cerevisiae) has emerged as a powerful model system for understanding molecular aspects of many human diseases. Protein misfolding linked to certain neurodegenerative diseases (NDDs) like Huntington Disease, Lou Gehrig's disease, and prion diseases have been successfully recapitulated in S. cerevisiae and led to identification of therapeutically relevant regulators of misfolding. No S. cerevisiae models for Parkinson's Disease (PD) or dentatorubral pallidoluysian atrophy (DRPLA) have been reported. PD is one of the most common NDDs, while DRPLA is a rare inherited NDD of the triplet repeat disease family. In both diseases, misfolding of a specific protein (alpha-synuclein for PD and atrophin for DRPLA) is thought to cause selective neuronal death. Unlike the well-characterized huntingtin protein in Huntington Disease (which shares many similarities to DRPLA), less is known about the misfolding of mutant atrophin in DRPLA. A S. cerevisiae expression system for studying alpha-synuclein has recently been developed in our lab. Preliminary evidence supports that both wildtype and disease-associated mutants are aggregating within yeast cells and upon purification. A similar effort to establish atrophin-1 expression in yeast is underway. To extend initial observations with alpha-synuclein in yeast and fully develop a yeast model for atrophin, three goals are proposed. 1) Misfolding properties between wildtype and mutant versions of both proteins will be investigated in vivo (immunofluorescence and GFP-based localization and assessment of protein half-life) and in vitro (by measuring protease sensitivity and differential solubility). 2) Influences of chaperones and ubiquitin-proteasomal pathway proteins on folding and degradation of these proteins will be assessed in strains compromised for chaperone/proteasomal function, or those that overexpress chaperones, and by co-immunoprecipitation assessment. 3) A fission yeast (S. pombe) expression model for alpha-synuclein and atrophin properties (as in Aim 1) will be developed and compared with the S. cerevisiae model; NDD models have not been reported in S. pombe. These studies may further clarify the molecular bases for misfolding and degradation of PD- and DRPLA-linked proteins and extend the usefulness of yeast models. Importantly, the scientific training of many undergraduates will be supported, strengthening their cell biology and molecular genetics skills and appreciation for model organisms. -

Principal Investigator: DICKSON, DENNIS W
Grant Number: 2P50NS040256-06
Title: Genetics and Molecular Biology of Parkinsonism

Abstract: The Udall Center for Excellence in Parkinson's Disease Research at the Mayo Clinic is an integrated, multidisciplinary center that studies the Genetics and Molecular Biology of Parkinsonism. The Center draws upon the clinical strengths of the Mayo Clinic Movement Disorder Section as well as epidemiologic and longitudinal studies of Parkinson's disease (PD), dementia with Lewy bodies and aging that provide clinical material for research projects. The Clinical Core is a multi-national effort to identify and characterize multiplex families with PD for genetic studies of PD. The Clinical Core also recruits and follows sporadic PD patients and arranges for postmortem studies. The Genetic Core provides genetic screening and performs genome wide linkage studies of familial PD. When permission is granted, samples are submitted to the NINDS DNA repository. The Neuropathology Core performs postmortem evaluations of PD, provides histologic support for projects and provides postmortem material collected through several different avenues for the research projects. Project 1 builds upon progress from the previous funding period demonstrating multiplication of the alpha-synuclein gene (SCNA) in autosomal dominant, early-onset PD and focuses on population genetics of SNCA, characterization of SNCA multiplications (including the size and genes within the multiplication regions), and measuring temporal and regional alpha-synuclein expression in normals and a-synucleinopathies. Project 2 is a clinicopathologic study that determines the frequency and clinical expression of Lewy bodies in normal individuals using the Mayo Medical Records Linkage System, with studies on the role of neuronal loss, inflammation and tau on clinical features. Project 3 uses cell lines that inducibly express alpha-synuclein as well as mitochondrial toxins, such as rotenone, to study truncated and aggregated alpha-synuclein with the goal of determining the role of interacting proteins in aggregate formation and the effects of aggregates on proteasome function and gene expression.-

Principal Investigator: DRISCOLL, MONICA A
Grant Number: 5R01NS041632-04
Title: C. elegans transporters in neuronal function

Abstract: Glutamate is an excitatory neurotransmitter essential for function of the nervous system. Certain injurious conditions cause excess glutamate to accumulate at the synapse, resulting in hyperstimulation of the post-synaptic neuron that can cause cell death. Glutamate transporters play an essential role in neuronal health and function by removing excess glutamate from the synaptic cleft. Considerable evidence implicates defective glutamate transport in ALS. It is particularly striking that approximately 65% of sporadic ALS patients have been reported to express aberrant glutamate transporter transcripts in affected neurons, a phenotype correlated with inhibition of glutamate transport. We propose to conduct a thorough analysis of glutamate transporter mutations in the facile C. elegans model system. The genome of this animal (sequenced to completion) encodes six glutamate transporter genes. We will determine when, and in which cells, the transporters are expressed, we will characterize their biochemical properties, and we will determine the loss of function phenotypes of each. We will also analyze the effects of expressing aberrant transporter transcripts, analogous to some produced in ALS patients, in transgenic animals. Finally, we will exploit the full power of C. elegans genetic analysis to conduct screens for new mutations that reverse or modify transporter defects. Genes identified in these screens should advance understanding of both normal and aberrant glutamate signaling. Since the C. elegans model system offers several unique advantages and most biological processes are conserved, we expect that results of the proposed study should provide new insight into basic mechanisms of Glu regulation at the synapse and may suggest novel strategies for preventing neurodegeneration in ALS. -

Principal Investigator: DURING, MATTHEW J
Grant Number: 1R01NS044576-01
Title: Somatic Cell Gene Transfer/Neurological & Clin Applics

Abstract: Gene transfer in the mammalian nervous system has been the primary research focus of our laboratory for the past decade. We are excited that this RFA has come at a time when the field is flourishing, yet clinical translation remains daunting, and much work needs to be done for ultimate success in the clinic. In this grant application we propose to focus on some of the more pressing needs using rat models of Parkinson Disease. Our first aim is to further develop more efficient and readily packaged and purified AAV vectors for clinical translation. Here, we will characterize and compare pseudotyped and chimeric AAV vectors and in addition develop novel reagents, including helper plasmids and protocols which can be used by the entire gene therapy community to more efficiently generate these vectors. Our preliminary data suggests that these new chimeric and pseudotyped vectors represent a significant advance above our current generation rAAV-2 vectors. Secondly, we will develop optimal expression cassettes with a focus on promoter; post regulatory sequences as well as elements like the human beta-interferon scaffold attachment region (SAR) to boost expression. Thirdly, we will further develop a regulatable system. We present in our preliminary data our latest generation bi-directional tet cassette with tandem minimal insulator sequences flanking the vector genome. Here we propose to use this vector as the starting point to develop a novel cassette with the use of KRAB-AB domain from kid-1 as a suppressor. Our fourth aim is the use of rAAV to over express PAEL receptor in the adult rat substantial nigra with characterization of the phenotype as a potential genetic model of Parkinson Disease. Finally, we propose the use of a picospritzer and in vivo single unit recording to develop methods for focal and electrophysiological mapped neuronal gene delivery. We will target the substantia nigra pars compacta, using AAV expressing wildtype parkin, as a potential therapy for parkin mutation associated, autosomal recessive Parkinson Disease (AR-PD) as modeled by the PAEL receptor over expressing rats as developed in specific aim 4.-

Principal Investigator: EL-KHOURY, JOSEPH
Grant Number: 5K08NS041330-04
Title: Chemokines and microglia in Alzheimer's disease

Abstract: The senile plaque is a pathological hallmark of Alzheimer's disease (AD). It is composed of beta amyloid fibrils (fAbeta), activated microglia, astrocytes and degenerating neurons. Data from patients and animal models of AD indicate that accumulation of microglia in senile plaques contributes significantly to neuronal degeneration. A key step is the migration of microglia to sites of fAbeta deposition. A likely course of events includes: (1) local microglia and astrocytes bind to fAbeta deposited in their vicinity, (2) this induces them to produce chemoattractants that recruit additional microglia, (3) recruited microglia adhere to fAbeta, become activated to produce neurotoxins, and other inflammatory mediators that cause neuronal damage. (4) Activated microglia are then retained in the senile plaques and continue to produce neurotoxins. We propose to investigate the mechanism of recruitment, activation and retention of microglia in senile plaques in AD. For this purpose we will use: (a) an in vitro model for microglial and astrocyte interactions with fAbeta, (b) post-mortem human brain specimen of patients with AD, and (c) transgenic APP mice (Tg2576) that develop AD-like pathology. We propose three specific aims: Aim 1. Identify chemoattractants produced by microglia and astrocytes interacting with fAbeta and determine their mechanism of production. We have preliminary data indicating that two chemokines, MCP-1 and fractalkine, can mediate migration of microglia to sites of fAbeta deposition in vitro. Aim 2. Study the effect of MCP-1, fractalkine and other chemoattractants; identified in aim 1 on key microglial functions important in the pathogenesis of AD. We will determine the role(s) of these chemokines in fAbeta-mediated activation of microglia to produce neurotoxins, and in microglial retention at sites of fAbeta deposition. Aim 3. Analyze the effect of targeted disruption or upregulation of MCP-1, CCR2, fractalkine or CX3CR1 on AD-like pathology in transgenic APP mice Tg2576. We will cross breed mice with targeted disruption or up-regulation of MCP-1, CCR2, and fractalkine genes with transgenic APP mice Tg2576 and test the resultant double transgenic mice for markers of AD. Chemokines and their receptors are attractive therapeutic targets in many inflammatory processes. Understanding the role of chemokines in recruitment and activation of microglia in AD may lead to exciting novel therapeutic targets to delay or stop the progression of AD by delaying or inhibiting the accumulation or activation of microglia at sites of deposition. -

Principal Investigator: FEANY, MEL B
Grant Number: 5R01NS041536-04
Title: Drosophila Model of Parkinson's Disease

Abstract: Parkinson's disease is a common neurodegenerative syndrome characterized by loss of dopaminergic neurons in the substantia nigra, formation of filamentous intraneuronal inclusions (Lewy bodies), and an extra pyramidal movement disorder. Although several genes involved in familial Parkinson's disease have recently been identified, we still know very little about the molecular and biochemical events mediating neuronal dysfunction and death of dopaminergic neurons. To enable a comprehensive genetic analysis of Parkinson's disease, we have developed a Drosophila melanogaster model of the disorder. Expression of human a-synuclein in transgenic flies replicates the three cardinal manifestations of the human disease: adult-onset loss of dopaminergic neurons, filamentous intraneuronal inclusions containing a-synuclein, and progressive locomotor dysfunction. We now propose to exploit the genetic potential of the system by generating second site suppressors and enhancers of a-synuclein mediated neurodegeneration. A robust and titratable retinal phenotype suitable for genetic modification has been defined. Existing collections of well-defined mutant chromosomes will he assayed for their ability to modify the retinal phenotype. De novo mutations will also be generated and tested. Mutations that modify the retinal phenotype will be tested for their ability to alter dopaminergic neurodegeneration and inclusion formation. Modifiers of neurodegeneration and inclusion formation will be characterized molecularly. Mammalian homologues of these Drosophila modifiers will he human disease gene candidates and likely components of mammalian neurodegenerative pathways. We will also test the role of the ubiquitin/proteosome system, chaperones, and apoptosis in dopaminergic neurodegeneration using genetic methods. The role of the ubiquitin system and heat shock proteins will also be tested by looking for the presence of these proteins in Drosophila asynuclein aggregates. Ubiquitin co-localization studies will further address the relevance of the Drosophila system to human disease, because ubiquitination is a pervasive feature of human Lewy bodies. We can abolish inclusion formation in a-synuclein transgenic flies, and will determine if inclusions are required for neurotoxicity. -

Principal Investigator: FOROUD, TATIANA M
Grant Number: 2R01NS037167-06
Title: Parkinson Disease Collaborative Study of Genetic Linkage

Abstract: In 1998, in response to an NIH invitation (PA-96-050) for investigator-initiated research on genetic factors that may be important in the development of Parkinson Disease (PD) a collaborative research effort to identify susceptibility genes for PD was established between Indiana University and investigators currently at the University of Rochester, the Parkinson Study Group (PSG), the University of California, San Diego, Cincinnati Children' s Hospital Medical Center, and the University of California, Irvine. During the first 4.5 years of this grant award, 422 multiplex PD families with 550 affected sibling pairs were recruited and rigorously evaluated. These genetically informative families were used to examine the role of the parkin gene in familial Parkinson's disease. Additionally, a genome screen was completed and evidence of linkage to chromosomes 2, 10 and X was consistently found in these family-based samples. During the next 5 years, we propose to extend our current results to: 1) continue to ascertain multiplex families with PD and thereby increase our power to detect and isolate PD susceptibility genes; 2) further examine the role of the parkin gene in PD through careful clinical evaluation of families with known parkin mutations; 3) identify the genes contributing to PD susceptibility in our multiplex families; and 4) test the role of any putative candidate genes identified through our family-based studies in a sample of sporadic PD cases and unaffected controls. In this way, we will expand the understanding of the etiology, pathogenesis, diagnosis, and ultimately, the prevention of this disease. To accomplish these goals we will focus on the following Specific Aims: 1. Expand the family resources so as to increase the power to identify the genes contributing to PD susceptibility. 2. Study the role of parkin mutations in Parkinson's disease. 3. Perform molecular studies to identify PD susceptibility genes. 4. Evaluate putative PD susceptibility genes in a case-control sample. -

Principal Investigator: GELLER, ALFRED I
Grant Number: 5R01NS043107-04
Title: Enhanced HSV-1 Vector Particles for Neural Gene Therapy

Abstract: This laboratory has developed a helper virus-free Herpes Simplex Virus (HSV-1) plasmid vector system for gene transfer into neurons. Using this system, we have begun to explore gene therapy approaches to specific neurological disorders, such as Parkinson's Disease (PD). We have shown that delivery of a HSV-1 vector that expresses human tyrosine hydroxylase into the partially denervated striatum in the 6-hydroxydopamine rat model of PD results in significant (64 percent) and long-term (1 year) behavioral recovery. Modifications to the vector particle have enhanced the utility of specific vector systems. First, the titers and infectivity of classical retrovirus vectors, lentivirus vectors, and other vector systems have been enhanced by pseudotyping with vesicular stomatitis virus (VSV) G protein. Recently, both we and other investigators have shown that HSV-1 vectors can be pseudotyped with VSV G protein and such vector particles can support gene transfer into neurons in the rat brain. Second, gene transfer has been targeted to specific types of cells by modifying the vector particle of classical retrovirus vectors or adenovirus vectors. Third, we have enhanced neural gene transfer and long- term expression by packaging vectors in the presence of mutations in specific HSV-1 proteins that affect the virion. The long-term goal of this proposal is to modify the HSV-1 vector particle to enhance its utility for human gene therapy of neurological disorders such as PD. The first specific aim will develop procedures for producing high titer HSV-1 vectors pseudotyped with VSV G protein. The second specific aim will target gene transfer to nigrostriatal neurons by modifying the HSV-1 vector particle to bind to specific receptors on these neurons. The third specific aim will enhance gene transfer and long-term expression by packaging vectors in the presence of mutations in specific HSV-1 proteins that affect the virion. These modified vector particles will be systematically characterized and then evaluated for gene transfer and expression in the rat brain. -

Principal Investigator: GESCHWIND, DANIEL H
Grant Number: 5R01NS040752-04
Title: THE GENETICS OF IDIOPATHIC BASAL GANGLIA CALCIFICATION

Abstract: The core features of idiopathic basal ganglia calcifications (IBGC) or Fahr's disease are dystonia, parkinsonism and neurobehavioral abnormalities that are associated with calcifications visible on CT scan of the brain. Familial IBGC shows mostly an autosomal dominant mode of inheritance. The investigators have mapped a locus on chr.14q in one large multiplex family. In two other families linkage to chr.14 has been excluded, demonstrating genetic heterogeneity. The minimal critical region (MCR) on chr.14 is 15 or probably 10cM. The investigators propose to narrow down the MCR by collecting additional family members of the original pedigree as well as other families. Physical mapping and candidate screening for mutations will be pursued as the region is narrowed to identify the IBGC gene. A genome scan will be performed in families who are not linked to the chr.14 locus. -

Principal Investigator: GILBERT, JOHN R
Grant Number: 5R01NS043473-03
Title: Genetics and Epidemiology of Essential Tremor

Abstract: Essential Tremor (ET) is a heterogenous tremor disorder characterized by a core group of features. The tremor syndrome is characterized by postural and kinetic tremor affecting the arms and hands, although the head, voice, and legs may also be affected. Although frequently described as a benign disorder, this is not true; many patients are socially and physically handicapped, with some patients being totally disabled. The differential diagnosis list for ET is extensive including dystonia, Parkinsonism, myoclonus, peripheral neuropathy, and other conditions. Prevalence estimates range widely, depending upon methodology and diagnostic criteria, from 0.003 to as high as 2% in the general population, with as much as 5% of the population affected over the age of 65. There are no known biological or diagnostic neuropathological markers for ET. The estimates of ET cases presenting with a positive family history range from 17.4% to 100%. Recent studies indicate that up to 96% of ET may be dominantly inherited. Clinical and genetic heterogeneity have slowed linkage studies. To date three loci associated with ET have been linked: 1) Familial Essential Tremor 1 (FET1) has been mapped in a series of Icelandic families on chromosome 3q13; (2) ETM mapped, in four unrelated US families, to chromosome 2p22-p25; and (3) a third locus maps, in a family that segregates both Parkinson's disease and postural tremor consistent with ET, to Chromosome 4p. We have, to date, ascertained, twelve ET and ET/PD linkage quality families. The largest pure ET kindred (DUK13001) have been excluded from known ET loci. The aims of this proposal are to ascertain and sample large families with ET, carry out a complete ET genome scan to establish linkage for these and additional ET families, identify new ET disease loci, and isolate and characterize ET genes, beginning with DUK13001 ET family.-

Principal Investigator: GLANZER, JASON G
Grant Number: 5F32NS046894-02
Title: Single-cell expression profiling of primary astrocytes

Abstract: Recent evidence has shown that astrocytes, a subset of glia, are capable of introducing and propagating calcium waves in vitro. Astrocyte processes are in close contact with synapses and can alter synaptic activity through regulation of glutamate in the perisynaptic space. The ability of astrocytes to propagate waves and alter synaptic activity suggest that these cells play active roles in brain signaling. Currently, astrocyte characterization has been based on morphological studies, whereas expression profiling of neurons has resulted in distinct classifications of cells based on the genes they express, which is often related to localization and function within the brain. Astrocyte subtypes may also exist in coordination with their neuronal partners. Therefore, we hypothesize that distinct astrocyte expression subtypes exist in the brain that are important for proper brain signaling and function. Using single-cell RNA amplifcation and DNA-array methods, we intend to develop an expression profile for primary astrocytes from different locations of the brain. Our laboratory has been successful in detecting both active translation and transcript specificity in dendrites. Likewise, we intend to identify the presence of active translation in astrocyte processes and identify what subset of mRNA transcripts are localized to the astrocyte processes. Expression profiling of astrocyte and astrocyte processes will provide a benchmark for future studies in characterizing these cells and may provide insight on pathological conditions thought to involve astrocyte dysfunction, such as multiple sclerosis, Parkinson's disease, and glioma.-

Principal Investigator: GOW, ALEXANDER
Grant Number: 5R01NS043783-03
Title: Molecular Mechanisms in Neurodegeneration

Abstract: The long term goals of this research are to define and characterize the molecular mechanisms by which missense mutations in proteins cause neurodegenerative disease. The accumulation of unfolded protein intermediates in various subcellular compartments is thought to underlie pathogenesis for a number of neurodegenerative diseases, including Alzheimers disease. A critical component of this research is the availability of patient data and well-defined animal models in mice so that full use can be made of the extensive tools available for genetic manipulation, such as gene ablation by homologous recombination and the introduction of heterologous transgenes. In Specific Aim number 1 an hypothesis that neurodegeneration stems from the accumulation of misfolded proteins in the endoplasmic reticulum will be tested in greater detail in vitro. These data will be correlated with disease severity in patients as determined by detailed clinical evaluation and in vitro transfection assays. Furthermore, autopsy specimens will be characterized at the levels of RNA, protein and immunocytochemistry to determine if pathogenesis in humans is similar to that in the animal models. In Specific Aim number 2 the pathogenesis of mutant mice, for which signaling pathways normally activated by protein accumulation have been disrupted in knockout mice, will be characterized in detail both molecularly and morphologically to provide a deeper understanding of the involvement of protein misfolding in neurodegenerative disease. In Specific Aim number 3 a number of important genes and proteins recently found to be activated by protein accumulation in the endoplasmic reticulum will be examined at the levels of RNA, protein and immunocytochemistry in mouse models of neurodegenerative disease to determine if protein misfolding activates similar signaling pathways to those identified by other investigators studying different cell types in in vitro systems. Furthermore, the identification of downstream target genes using microarray screens will be sought to identify and characterize the signaling pathways that are activated by unfolded proteins, and ultimately determine if a cell survives or dies. Together, these Aims are expected to identify important pathological processes stemming from protein accumulation and may lead to strategies that ameliorate disease severity. Moreover, the knowledge gained from these studies may be applicable to the amelioration of other neurodegenerative diseases for which protein misfolding is a cause but the genetic or metabolic defects are unknown.-

Principal Investigator: GROSS, ROBERT E
Grant Number: 1K08NS046322-01A1
Title: Axon Guidance Molecules in Nigrostriatal Regeneration

Abstract: We are interested in developing strategies for the reconstitution of the dopaminergic (DA) nigrostriatal (NS) pathway that degenerates in Parkinson's disease, an important goal because of the inadequacy of current long-term treatments. Attempts to reconstruct this pathway through transplantation of precursor cells or neurons into the nigra of the adult fail, likely as a result of 1) the presence of inhibitory molecules and/or 2) the absence of trophic and guidance molecules in the adult CNS. Here we propose that an understanding of the molecular events that regulate the development of the nigrostriatal pathway will provide insights for strategies designed to improve NS pathway regeneration in the adult milieu. We propose - and have exciting preliminary data to support - that axon guidance molecules (AGMs), important molecules that direct the development of other projection pathways in the CNS, are expressed in the developing DA NS pathway. A series of experiments are proposed to elucidate the role played by AGMs and their receptors in the development of the NS pathway. Our specific aims are to: 1) Define those AGMs whose receptors are expressed in the developing axons of nigral DA neurons; 2) Define the expression of AGM ligands in relation to the developing NS pathway; 3) For those AGMs that are expressed in an appropriate anatomical relationship to influence NS development, and whose receptors are expressed in developing DA neurons, directly demonstrate chemotropic effects on fetal nigral DA neurons in vitro, and their importance in the development of the NS pathway with blocking studies ex vivo. The outcome of the experiments outlined in this proposal will hopefully be the refinement of means to counteract the inhibitory milieu of the adult injured nervous system, and recapitulate the attractive and repulsive factors that direct axonal outgrowth during development, thereby paving the way for novel reconstructive and regenerative strategies to ameliorate the symptoms of Parkinson's disease. The insights derived from these studies may also have applicability in other neurodegenerative diseases, brain injury and stroke. The research outlined is part of a customized five-year plan of training and career development for the Principal Investigator. The proposal includes active mentoring by experienced scientists, access to diverse resources, and an environment uniquely suited to help the PI develop as an independent neurosurgeon-neuroscientist. -

Principal Investigator: GUO, SU
Grant Number: 5R01NS042626-02
Title: Development of Dopaminergic Neurons in Zebrafish

Abstract: Dopaminergic (DA) neurons synthesize and release neurotransmitters dopamine. The importance of DA neurons is underscored by their involvement in multiple human neurological disorders, for instance, Parkinson's disease. Despite their functional significance, the mechanisms determining the development of these neurons are not well understood. Elucidation of these mechanisms is essential to defining and interpreting the causes of disorders affecting DA neurons and developing regenerative therapy for treating Parkinson's disease. Meanwhile, understanding the development of DA neurons will also shed light on fundamental mechanisms governing cell identity and diversity and neural circuit formation in the vertebrate nervous system. The long-term goal of this project is to understand the molecular mechanisms that control the identity and connectivity of subtypes of DA neurons in vertebrates. We are taking a genetic approach in zebrafish, a vertebrate model organism that offers a unique combination of excellent genetics and embryology. We have localized major DA neuronal subtypes in developing zebrafish. By carrying out a genetic screen based on immunohistochemistry, we have identified mutations in three genes that are required for proper development of subtypes of DA neurons. Molecular cloning of the foggy gene revealed the importance of regulated transcription elongation in DA neuron development. Thus, we shall explore how this previously under-appreciated mode of gene regulation is involved in DA neuron development. Phenotypic analysis suggested that the motionless and twin-of-motionless mutations disrupt a signal important for DA neuron induction. Therefore, their molecular identity will be determined. By analysis of cloned genes and existing mutations, we will identify essential machinery involved in controlling DA neuron development. These molecules will not only provide important insights into vertebrate neural development, but may also help develop regenerative therapy for treating neurological disorders such as Parkinson's disease. -

Principal Investigator: Higgins, Joseph J.
Grant Number: 5R01NS039353-05
Title: POSITIONAL CLONING OF A GENE FOR ESSENTIAL TREMOR

Abstract: Essential tremor (ET), the most common movement disorder in humans, significantly compromises the livelihood or social function of at least 85 percent of the 4 million individuals affected with the disease in the United States. Aggravated by emotions, hunger, fatigue and temperature extremes, the condition may cause a functional disability or even incapacitation. The main clinical feature of ET is postural tremor of the arms, but the head, legs, trunk, voice, jaw, and facial muscles also may be involved. The majority of cases are familial and the disease is usually an autosomal dominant trait with incomplete penetrance. The identification of two susceptibility loci on chromosomes (chr) 2p22-p25 (ETM) and chr 3q13.1 (FET1) implies that ET is genetically heterogeneous. We originally identified the ETM locus in a single American family of Czech descent with pure ET, and later refined the location of the ETM gene to 9.1 centiMorgan region by genotyping three additional families with a similar phenotype. The long-term objectives of the proposal are to identify the other ET susceptibility loci by linkage analysis and to characterize these genes by positional cloning techniques. The specific aims are the following: 1). Collect additional individuals and families with ET. 2). Define the minimal critical region (MCR) that contains ET genes by identifying key recombinants. 3). Construct a high-resolution physical map (contig) of the MCR. 4). Isolate the genes within the contig and evaluate these candidates for disease-causing mutations. The results of this research will enhance our understanding of the human motor system in general and the pathogenesis of tremor in particular. Because current pharmacological treatments for ET have limited efficacy and often become ineffective with advancing disease, identifying the genes that cause ET will facilitate the development of more effective therapeutic strategies. -

Principal Investigator: HUNG, ALBERT Y
Grant Number: 5K08NS041411-04
Title: Activity-Dependent Regulation of Synapses by Shank

Abstract: The goal of this project is to investigate the role of a newly discovered postsynaptic protein, Shank, in the regulation of dendritic spine morphology and cytoskeleton. Local electrical stimulation induces growth of dendritic spines, suggesting that synaptic activity directly modulates neuronal architecture and circuitry. The molecular basis for these activitydependent changes is not known, but probably involves postsynaptic proteins that interact with receptors and/or cytoskeletal elements. Shank acts as a putative scaffold for multiple glutamate receptor subtypes and also binds to the actinbinding protein cortactin, which has been implicated in dynamic cytoskeletal rearrangement and translocates to synapses in response to glutamate. This study examines the role of Shank in the regulation of dendritic spines and its in vivo function through three specific aims. First a combination of cell biological, biochemical, and dominant inhibitory approaches will be used to determine the mechanism for glutamateregulated cortactin translocation to synapses, and to identify if Shankcortactin interaction is required for this response. Second, how Shank induces spine growth will be studied by structurefunction analysis. Finally, a genetic approach, generation of a Shank1 "knockout" mouse, will be used to investigate the role of Shank proteins in brain development, in postsynaptic receptor organization, and in learning and memory. The longterm goal of the candidate is to understand how aberrant synaptic transmission contributes to neurologic disease. Synapses are the signal processing units of the brain, and overexcitation of synapses by glutamate is thought to play a role in both acute neuronal injury (such as stroke and seizure) and chronic neurodegenerative conditions (including Huntington's disease, Parkinson's disease, and amyotrophic lateral sclerosis). Understanding how postsynaptic proteins, such as Shank, regulate activitydependent synaptic plasticity may shed light on mechanisms of glutamate toxicity. The immediate goal is to obtain training in the most uptodate techniques in molecular genetics, protein biochemistry, and cellular neurobiology, sponsored by Dr. Morgan Sheng, which will enable him to become a productive, independent molecular neurologist. -

Principal Investigator: HUTSON, CHE B
Grant Number: 1F31NS051163-01
Title: The Role of Inflammation in Parkinson's Disease

Abstract: Parkinson's disease (PD) is a neurological disorder characterized by the degeneration of nigrostriatal dopaminergic neurons. The cause of this degeneration has yet to be fully understood. However, there is increasing evidence that PD is the result of a complex set of interactions encompassing genetic predisposition, the innate oxidative characteristics of the nigrostriatal dopaminergic pathway and inflammation. Less than 10% of PD cases are hereditary. A subset of which has been linked to two mutations in the alpha-synuclein gene. Our laboratory has obtained a mouse that over-expresses human alpha-synuclein under the control of the platelet-derived growth factor promoter. Using this mouse as a genetic model of PD, I plan to examine the inflammatory mechanisms leading to the loss of nigrostriatal dopaminergic neurons after exposure to the inflammagen lipopolysaccharide (LPS). I hypothesize that in the context of increased alpha-synuclein expression, inflammation is detrimental to dopaminergic neurons. Furthermore, I hypothesize that LPS mediated inflammation will result in the loss of dopaminergic cells in the substantia nigra of the alpha-synuclein over-expressing murine model of PD. -

Principal Investigator: IACOVITTI, LORRAINE M
Grant Number: 2R01NS032519-11A1
Title: Studies of Purified Dopamine Neurons

Abstract: Historically, there has been no good way to isolate DA neurons from other cells of the midbrain. Thus, missing DA neurons have been replaced by mixed cell populations following transplantation of embryonic midbrain tissue in animal models of disease and in Parkinson's patients. Although, in many cases, these transplants have provided long-term benefit, the presence of unwanted cells, such as glia, non-DAergic neurons, or even excessive numbers of DA neurons, has produced serious side effects, and in rare cases, even death. Discovering ways in which to segregate DA neurons from other cell types poses a significant challenge, but a necessary next step. In the present proposal, our plan is to take advantage of several new advances in the laboratory; including the recent cloning of 11kb human tyrosine hydroxylase gene promoter (hTH). This sequence accurately targets the expression of the reporter, green fluorescent protein (GFP) to DA neurons of the mammalian CNS. Because GFP can be directly visualized in live fetal DA neurons, this approach allows enrichment via flourescent activated cell sorting (FACS) for study in vivo and in vitro. Moreover, it is possible to adapt these purification methods to mouse stem and human progenitor cells using a lentiviral vector to transduce cells with the hTH-GFP transgene. Following their DA differentiation and FACS sorting, our goal is to study purified populations of engineered stem/progenitor-derived DA neurons in culture or after transplantation into the Parkinsonian rat. These models offer us a unique opportunity to determine the ideal number of DA neurons needed as well as the optimal conditions which contribute to their survival and growth following transplantation. Graft function will be assessed in live animals via behavioral testing and in vivo microdialysis which will be correlated with biochemical and anatomical (at the light and electron microscopic levels) changes following sacrifice. This work will hopefully lay the foundation for the development of therapeutic treatments for Parkinson's and other diseases involving compromised DA systems. -

Principal Investigator: KAPATOS, GREGORY
Grant Number: 2R01NS026081-15A1
Title: Tetrahydrobiopterin Biosynthesis by Dopamine Neurons

Abstract: GTP cyclohydrolase I (GCH1) catalyzes the first and rate-limiting step in the synthesis of tetrahydrobiopterin, the essential cofactor for tyrosine hydroxylase and the production of dopamine (DA) within nigrostriatal DA (NSDA) neurons. A complete analysis of the cis-acting elements in the GCH1 proximal promoter necessary for basal and cAMP-dependent transcription is near and the goal of Aim 1 is to identify cognate binding proteins for the GC-box and determine their role in basal and cAMP-dependent GCH1 transcription. The trans-acting factors recruited by these c/selements are also important and the goal of Aim 2 is to forge a link between phosphorylation of C/EBPbeta and NF-Y and cAMP-dependent GCH1 transcription. Little is known about how NSDA neurons regulate GCH1 gene expression. The goal of Aim 3 is to understand the temporal changes in protein-promoter DNA interactions that take place during cAMP-dependent GCH1 transcription in NSDA neurons and to test the hypothesis that GCH1 transcription is negatively coupled to somatodendritic D2 autoreceptor tone. Heterozygous mutations in GCH1 can cause DOPA-responsive dystonia (DRD), an autosomal dominant disorder with partial penetrance that selectively decreases DA synthesis within NSDA neurons and presents in childhood as a dystonia and in adulthood as Parkinson's disease (PD). Half of DRD patients have no mutation in the GCH1 open reading frame and presumably have mutations in GCH1 gene regulatory regions. The conserved genomic cis-elements we have already described are therefore likely sites for mutations associated with GCH1 deficiency. Unaffected first-degree relatives of DRD patients are also known to have a 23-fold higher incidence of parkinsonism than do normal controls, suggesting a link between DRD, GCH1 and PD. With the hypothesis that background genetic variability in GCH1 may promote susceptibility to familial parkinsonism and idiopathic PD, we propose in Aim 4 to sequence and functionally characterize mutations in GCH1 proximal promoter and coding regions in familial parkinsonism. Because association mapping is potentially a more powerful strategy for identifying genetic variability additional studies in Aim 4 will assess genetic variability within the GCH1 gene in PD cases versus controls. The goal of Aim 5 is to determine whether genetic variability in the human GCH1 gene influences GCH1 transcription or GCH1 enzyme activity. We expect that this multidisciplinary approach will yield important new information on the role of GCH1 in NSDA neuron function and will lead to a new understanding of DRD and familial and idiopathic PD. -

Principal Investigator: KAPLITT, MICHAEL G
Grant Number: 1K08NS044978-01A2
Title: PTEN Anti-Oncogene: Neuronal Function and Toxicity

Abstract: The PTEN anti-oncogene is among the most frequently mutated genes in malignant brain tumors. Normally, PTEN is a lipid phosphatase which blocks malignant phenotypes primarily by inhibiting the PI3 Kinase/AKT pathways, but PTEN can also act as a protein phosphatase. PTEN is expressed in brain late in development, and neuronal expression continues throughout adult life. Although loss of PTEN can cause neuronal hyperplasia, little is known about the role of PTEN in neuronal development or in normal neurons. Pathways influenced by PTEN suggest that this anti-oncogene may increase neuronal sensitivity to toxicity and/or degenerative processes, which is supported by our preliminary data. This proposal will first determine whether PTEN can modulate sensitivity of cultured neuron-like cells to toxins used in models of Alzheimer's disease and Parkinson's disease. While studying this hypothesis, we have unexpectedly found that PTEN blocks NGF signaling in PC12 cells, and this appears to be at least partially due to inhibition of expression of trkA and p75 NGF receptors at the protein and mRNA levels. DNA microarray then revealed that PTEN can inhibit expression of several genes, including tyrosine hydroxylase and GTP cyclohydrolase 1. Since this may also have implications for neuronal function and for Parkinson's disease, the second Aim of this proposal will also explore the mechanism by which PTEN inhibits expression of these genes. The final Aim of this proposal will explore the effect of age and neurotoxins used in models of neurodegenerative disorders on PTEN levels and function to determine the biological relevance of data generated from the first two Aims. These studies and my development as an independent clinical scientist will be significantly advanced by Dr. M. Flint Beal, who will serve as my sponsor and who is a leading expert in neuronal degeneration in PD and AD. Additional mentoring by Dr. Eric Holland, a leading expert on anti-oncogene signal transduction, will also add significantly to my scientific growth and will also help me to realize many of the Specific Aims of this proposal. The environment at Cornell and the strong support of my institution will permit me to focus upon these studies with minimal distractions. My scientific background is substantial, and this will facilitate realization of the goal of this project. This plan outlined in this award will, however, enhance previously underserved aspects of my education while focusing on an important scientific question, in order to promote a successful transition to scientific independence.-

Principal Investigator: KIM, KWANG S
Grant Number: 5R21NS044439-02
Title: DA-specific gene discovery and promoter engineering

Abstract: Gene therapy techniques need substantial development to provide therapeutic possibilities for treating neurological disorders such as Parkinson's disease (PD). Based on molecular control mechanisms of noradrenergic neuron-specific gene regulation, we recently devised a gene delivery system that can efficiently target transgene expression to noradrenergic neurons in a cell-specific manner. Our long-term goal is to establish gene therapy system(s) that will drive efficient transgene expression in a dopamine (DA) neuron-specific fashion based on discovery and characterization of DA-specific genes. Toward this end, we propose to identify and isolate genes that are selectively expressed in the DA mid-brain area by analyzing gene expression profiles using the most comprehensive cDNA microarrays such as the augmented NIA 16K chip and augmented RIKEN 16 K chip. Because these chips do not cover the whole genome yet, we will also identify novel DA-specific genes by the PCR-based subtractive hybridization techniques. Expression patterns of putative DA-specific genes will be tested by semi-quantitative RT-PCR using independently isolated mRNAs, and will be confirmed by in situ hybridization. Among the isolated DA-specific genes, we will first focus on putative DNA-binding transcription factors. The consensus binding sites for these putative transcription factors will be defined and their potential promoter function will be tested by cotransfection assays using cell line systems. On the basis of the mechanism of action of the novel DA-specific transcription factor(s), synthetic promoters will be developed and optimized. The optimized synthetic promoter will be subcloned in front of the reporter lacZ gene in the context of the self-inactivated lenti viral vectors. Cell type-specific expression of the reporter gene will be examined using both in vitro mesencephalic primary neuronal cultures as well as in different rat brain areas following stereotactic injection. At the later stage of this proposal, we will plan to use our developed promoter system(s) to deliver therapeutic genes (e.g., GDNF and Bcl 2) to the DA neurons and will test whether they can efficiently ameliorate behavioral symptoms in animal models of PD. The proposed research will identify and isolate genes that are selectively expressed in the mid-brain DA area on a genome-wide scale and will characterize their transcriptional regulation. Based on these mechanisms, we will devise novel and innovative DA-specific promoter systems and test them using in vitro and in vivo systems. In combination with safe viral vectors, our developed gene delivery systems can be translated clinically into gene therapy approaches for PD and other neurological disorders, in which DA

Principal Investigator: KONRADI, CHRISTINE
Grant Number: 1R01NS048235-01
Title: Levodopa dyskinesia and striatal neuroplasticity

Abstract: Parkinson's disease (PD) is a brain disorder caused by progressive loss of the brain chemical dopamine. Patients with Parkinson's disease are treated with levodopa (L-DOPA), a precursor of dopamine. However, L-DOPA therapy has disabling side effects. Most patients on L-DOPA treatment are eventually afflicted with motor fluctuations and abnormal, involuntary movements known as dyskinesias. L-DOPA-induced dyskinesias can become more disabling than Parkinson's disease itself. In severe cases, neurosurgical lesioning of basal ganglia nuclei such as the thalamus, pallidum or subthalamic nucleus is needed to improve Parkinson's disease and to minimize L-DOPA dosage. The proposal is based on the hypothesis that L-DOPA treatment in Parkinson's disease, and L-DOPA-induced dyskinesia, are accompanied by unique patterns of gene expression in the putamen. By comparing the gene expression patterns of dyskinesia to non-dyskinesia, we may find the critical factors responsible for the development of dyskinesia, or responsible for preventing the development of dyskinesia. Specific therapies could then be devised that could be co-administered with L-DOPA to prevent dyskinesias. We propose to investigate the molecular systems that are altered in L-DOPA-induced dyskinesia, and to find the 'molecular signature' of dyskinesia. We will study gene expression patterns in the post mortem putamen in Parkinson's disease in response to L-DOPA treatment (PD; Specific Aim 1) and in response to L-DOPA-induced dyskinesia (Specific Aim 2), and compare it to a rat model of L-DOPA-induced dyskinesia (Specific Aim 3). The role of five candidate genes for the development of, or compensation for, dyskinesia will then be examined in the rat model (Specific Aim 4). In a gene array experiment we have already collected data from the rat model of dyskinesia and assembled lists of candidate genes from these data. The lists of genes will be cross-referenced with the findings in the human putamen to determine five most likely candidates to be tested in the rat model. Hypothesis testing will be combined with computer programs that can find interesting new, unanticipated patterns of gene regulation, and help to formulate new hypotheses. The post mortem samples provide us with direct access to the human condition, while the animal model provides us with an experimental system that can be tightly controlled and that permits functional analyses and hypothesis-testing. Together they can lead the way toward new treatments for dyskinesia. -

Principal Investigator: KONTOPOULOS, EIRENE
Grant Number: 1F31NS049869-01
Title: Mechanisms of Neurotoxicity in Parkinson's Disease

Abstract: Our long-term objective is to elucidate the underlying mechanisms of neuronal death in Parkinson's disease (PD). The identification of several genes exhibiting linkage to PD has not yet led to the understanding of how their protein products bring about cell death. Though in vitro studies have been instrumental in identifying potential mechanisms of neurodegeneration, their findings need to be corroborated in vivo. I propose to utilize Drosophila genetics to investigate putative protein interactions among three PD-linked genes: synphilin-1, alpha-synuclein, and parkin. My primary strategy will be to investigate the inherent toxicity of synphilin-1 and its PD-associated mutation, R621C. Furthermore, genetic interactions between both forms of synphilin and either alpha-synuclein or parkin will be examined. These efforts will culminate in the investigation of genetic interactions among synphilin-1, alpha-synuclein, and parkin. -

Principal Investigator: KOTZBAUER, PAUL T
Grant Number: 1K08NS048924-01
Title: Neurodegenerative consequences of PanK2 mutations

Abstract: The candidate is an M.D./Ph.D neurologist who is currently a trainee in the Center for Neurodegenerative Disease Research. His goal is to develop additional research skills and experience needed to become an independent clinician scientist working to understand the pathogenesis of neurodegenerative diseases. The proposed research project focuses on neurodegeneration with brain iron accumulation (NBIA), which causes progressive impairment of speech, movement and cognition. At the neuropathological level, NBIA is characterized by iron accumulation, inclusion formation, signs of oxidative stress, and death of multiple neuronal populations. These features are also seen to varying degrees in other neurodegenerative diseases, including Parkinson's disease and Alzheimer's disease. Mutations in the gene for pantothenate kinase 2 (PanK2) were recently identified in a subset of NBIA cases. The PanK2 gene encodes an enzyme involved in coenzyme A (CoA) synthesis, a critical pathway linked to a number of cellular processes, including fatty acid synthesis, energy production, and possibly, synthesis of anti-oxidant molecules. The long term objectives of this project are to understand how PanK2 mutations lead to iron accumulation, oxidative stress, inclusion formation, and neuronal death. The proteolytic processing, mitochondrial localization and in vitro catalytic properties will by characterized for mutant Pank2 proteins and compared to the wild type human PanK2 protein. Cell culture systems will be established in which PanK2 expression is eliminated and in which wild type or mutant PanK2 proteins are over-expressed. Mice that lack PanK2 expression will also be generated. Cell lines and mice lacking PanK2 expression will be examined for changes in levels of biochemical intermediates hypothesized to be dependent on PanK2 function. Finally, neuronal and non-neuronal cells lacking PanK2 will be examined for signs of increased oxidative stress, susceptibility to oxidative injury, cellular and mitochondrial import of radio labeled iron, and inclusion formation.-

Principal Investigator: KRAMER, HELMUT J
Grant Number: 5R01NS043406-03
Title: Hook proteins in membrane trafficking & neurogeneration

Abstract: Neurodegenerative diseases such as Huntington's disease, amyotrophic lateral sclerosis or Parkinson's disease share one common feature, the slow accumulation of misfolded proteins. As misfolded proteins accumulate in neurons they are not evenly distributed. Instead, they are concentrated in inclusion bodies. How these inclusion bodies are linked to the progression of neurodegenerative diseases is not well understood. One class of inclusion bodies, aggresomes, are formed at the microtubule organizing center in an active process that requires microtubule-based transport. We recently discovered that the active concentration of misfolded proteins in aggresomes involves the Hook2 protein. Hook proteins constitute a family of coiled-coil proteins which bind to microtubules and affect the organization of different organelles in mammalian cells and in Drosophila. In this grant, we will combine genetic approaches in Drosophila, cell biological approaches in mammalian tissue culture cells and biochemical experiments in-vitro to characterize shared functions of Hook proteins, as well as the specific role of Hook2 in the cellular trafficking of misfolded proteins. In Spec. Aim 1, we will determine the relevance of microtubule binding of Hook proteins using a combination of biochemical approaches in vitro and genetic experiments in Drosophila. In this context we will also explore the potential interaction of Hook proteins with the complex between cytoplasmic Dynein and Dynactin. In Spec. Aim 2, we will characterize the binding of Hook proteins to different organelles and identify the receptors that mediate these interactions. In Spec. Aim 3, we will determine the role of Hook2 in the formation of aggresomes and the potential of using dominant-negative forms of Hook2 to manipulate the aggregation of different misfolded proteins. In Spec. Aim 4, we will determine the domains of Hook proteins responsible for their polarized distribution in neurons and the role of Hook proteins in establishing neuronal polarity in rat hippocampal neurons. -

Principal Investigator: KURLAN, ROGER M
Grant Number: 1U01NS050095-01
Title: Parkinson's Disease Data Organizing Center

Abstract: In response to RFA-NS-NS-05-001, we propose to establish a Parkinson's Disease Data Organizing Center (PD-DOC) at the University of Rochester. In keeping with the RFA, the PD-DOC will: 1) establish, maintain and disseminate a shared, central and standardized longitudinal database in support of the prospective collection and analysis of clinical, neuropathological and biologic data from patients with PD and controls, 2) assess and move toward the potential integration of relevant pre-existing databases, 3) assist investigators planning to perform research studies using the shared database, 4) prepare and maintain an up-to-date catalog of research materials at participating sites that might be used for PD research and, 5) coordinate annual meetings of the PD-DOC Steering Committee. The University of Rochester has extensive expertise and resources which will facilitate the development of a highly successful PD-DOC. The PD-DOC will be a critical force in advancing collaborative research in PD. -

Principal Investigator: LANSBURY, PETER T
Grant Number: 3P50NS038375-05S1
Title: FAMILIAL PARKINSON'S DISEASE: CLUES TO PATHOGENESIS

Abstract: Unavailable

Principal Investigator: LANSBURY, PETER T
Grant Number: 1R21NS047420-01A1
Title: High Throughout Assay to Probe UCH-L1 Ligase Inhibitors

Abstract: Parkinson's disease (PD) is characterized by the presence of Lewy bodies (the cytoplasmic neuronal inclusions) and the significant loss of dopaminergic neurons in the substantia nigra, a-synuclein was identified as one major fibril component of the Lewy bodies, thus linked the accumulation of this protein to the pathogenesis of PD. Failure to regulate the concentration of a-synuclein, for example by dysfunction of the pathogenesis of PD. Failure to regulate the concentration of a-synuclein, for example by dysfunction of degradation process, can also contribute to the build-up and consequently fibrillation of the protein. A gene, PARK5, has been linked to PD are involved in proteasomal degradation pathway and it is an ubiquitin C terminal hydrolase (UCH-L 1) that hydrolyzes C-terminal ester and amides of ubiquitin and is believed to play a key role in processing polyubiquitin and/or ubiquity lated proteolytic peptide. A rare mutation (193M) of UCH L 1 that yields a 50% reduction in its hydrolytic activity has been tentatively linked to a rare early onset form of PD, at the same time a polymorphism of the enzyme (S 18Y) was indicated to reduce the risk of PD. The assumption that each enzyme expresses a single enzymatic activity in vivo, however, is challenged by the linkage of UCH-L 1 to PD. UCH-L 1, especially those variants linked to higher susceptibility to PD, causes the accumulation of a-synuclein in cultured cells, an effect that cannot be explained by its recognized hydrolase activity. UCH-L1 exhibits a second, dimerization-dependent, ubiquityl ligase activity. The polymorphic variant of UCH-L1 that is associated with decreased PD risk (S 18Y) has reduced ligase activity, but comparable hydrolase activity as the wild-type enzyme. Thus the ligase activity, as welt as the hydrolase activity of UCH-L1 may play a role in proteasomal protein degradation, a critical process for molecules ("molecular probes") that can be used to perturb UCH-L1 ligase activity in cell culture and animal models of PD. This "chemical genetic" strategy is complementary to traditional genetic approaches (e.g., knockouts and trasngenics) for understanding protein function but has a distinct advantage in that the probes are potential lead compounds for the development of novel PD therapetutics. The program detailed below will seek probes with the following activities: (1) inhibitors ofUCH-L1 dimerization, (2) inhibitors of UCH-L1 ligase activity, and (3) repressors and activators of UCH-L1 expression. -

Principal Investigator: LAURING, BRETT
Grant Number: 1R01NS043298-01A2
Title: Identification of Novel Alpha Synuclein Binding Protein

Abstract: Parkinson's Disease (PD) is the second most common neurodegenerative disease. As in many other neurodegenerative diseases, conformational alteration of a specific neuronal protein results in the accumulation of fibrillar amyloid inclusions, which in the case of Parkinson's disease, are termed Lewy Bodies (LBs). LBs have a fibrillar core with the fibrils being comprised primarily of a protein of unknown function called alpha-synuclein. Alpha-synuclein mutations cause autosomal dominant Parkinson's disease. Thus both human genetic and histologic evidence link synuclein to Parkinson's disease. Alpha-synuclein is a 140 aa protein which is 'natively unfolded' meaning that it has no identifiable secondary structure. However, in the presence of certain lipid membranes is can fold into a alpha helical conformation, and when incubated alone can fold into a beta-sheet rich conformation which allows it to form amyloid fibrils resembling those seen in Lewy bodies. Consistent with the hypothesis that alteration of synuclein conformation is linked to development of Parkinson's disease, purified mutant synuclein fibrillizes more rapidly than wild-type protein in vitro. Overexpression of synuclein as a transgene results in formation of Lewy body-like pathology in mice and flies. Synuclein expressed at endogenous levels rarely forms amyloid (only in PD patients), is not stably membrane-associated, and remains 'unfolded'. The discrepancy between the in vivo folding parameters and those observed in vitro leads us to hypothesize that synuclein-interacting molecules may regulate synuclein conformation, stabilize it in the 'unfolded' state, or regulate membrane binding. We therefore set up a novel photo-cross linking assay heretofore not used to study synuclein to identify synuclein binding proteins present in brain extracts and present at endogenous levels of expression to begin to determine how synuclein conformation is regulated. We have identified novel synuclein binders. We propose to develop a fluorescence resonance energy transfer assay capable of indicating synuclein conformation both in vivo and in vitro. That will allow for screening of proteins and synthetic agents capable of altering synuclein aggregation. These studies will enable us to define the range of proteins or agents to be further characterized in in vivo models of Parkinson's disease.-

Principal Investigator: LAWRENCE, MATTHEW S
Grant Number: 1R43NS048786-01
Title: Genomic markers of environmental toxins for Parkinsonism

Abstract: Parkinson's disease is a prevalent and devastating neurodegenerative condition of unknown etiology. One prominent hypothesis holds that the selective loss of the nigrostriatal dopaminergic neurons characteristic of the disease results from damage from environmental neurotoxins in genetically vulnerable individuals. Identifying such environmental contributors to Parkinson s pathogenesis represents a significant public health concern. This project aims to identify the in vivo gene expression changes that occur in the primate brain in response to environmental toxins that have been implicated in the production of Parkinson's and compare these changes with the selective neurotoxin, MPTP, and with the limited knowledge of genetic abnormalities in some Parkinson's patients. Because of the unique vulnerabilities of nonhuman primates and humans to dopamine neurotoxic agents, studies in primates are essential to uncover common genetic markers of toxicity and to reveal the potential toxicity of chemicals of unknown liability. The proposed Phase I studies will test the hypotheses that transcriptional changes that accompany and precede dopamine cell death can be identified using high density gene arrays and bioinformatics in the primate nigrostriatal system in vivo following MPTP exposure. Changes in mRNA initiation of regimen of 3 doses of MPTP over 36 hours that has been established to result in Parkinsonism. Expression changes will also be assessed 6 hours after the administration of a single dose. Changes in nigrostriatal dopamine concentrations and tyrosine hydroxylase immunohistochemistry will be assessed at all time points. Additionally neurobehavioral changes will be assessed in the 20-day animals. Together these data will allow a determination of the sequence of transcriptional changes that parallel or precede histological, biochemical and behavioral events, and allow an assessment of transcriptional events related to acute versus chronic toxicity, with confirmation by quantitative RT-PCR. Defining the chronological and dose dependent gene expression changes induced by MPTP may reveal a transcriptional profile that is predictive of nigrostriatal injury from this toxin. Phase II studies will address whether similar gene expression changes and neuronal injury are seen following exposure to environmentally prevalent compounds that are postulated to be risk factors for the development of Parkinson's disease, and to integrate the resulting transcriptional data into a toxicogenornic database and potentially customized microarrays which may be applied to the assessment of compounds for their possible health risk.-

Principal Investigator: LE, WEI-DONG
Grant Number: 5R01NS043567-02
Title: NURR1 Gene Mutations in Parkinson's Disease

Abstract: The search for genes that cause or contribute to Parkinson's disease (PD) has intensified since the discovery of alpha-synuclein gene and parkin gene mutations linked to patients with familial Parkinson's disease. NURR1 {the human homolog of roden nurr1), a member of the nuclear receptor super family, may be relevant to the disease since the gene is essential for induction and maintenance of nigra dopaminergic (DAergic) adenotype and heterozygous nurr1 knock-out mice display many features of parkinsonism with aging. To investigate if NURR1 is a susceptibility gene in Parkinson's disease we have performed a case-control study in over 200 PD patients. We have identified two novel variants in NURR1 gene (-291T deletion and -245T ->G substitution) in 10 of 107 proband familial Parkinson's disease (fPD), but not in sporadic PD (sPD, n=94) and age-and race-matched normal controls (NC, n=221). In pedigree analysis of the ten fPD families we have found that all PD patients but none of the non-PD family members have the NURR1 gene variations. A linkage study using 5 haplotype markers in 4 available fPD families suggested that at least two distinct haplotyes exist in these fPD families. Furthermore, we have constructed full-length human NURR1 gene encoding the two variants and demonstrated that the variant genes reduced NURR1 gene expression by >85% when transfected in human embryonic kidney cells HEK293 or in human neuroblastoma cells SH-5YSY. In addition, rate-limited DA synthesis enzyme tyrosine hydroxylase mRNA was significantly low in the SH-5YSY cells transfected with two variant genes. These data suggest that the variants in the NURR1 gene might be PD-related mutations. We, therefore, hypothesize that diminished expression of the NURR1 gene (loss of function), induced by the mutations in the gene, predisposing to DAergic neuron dysfunction, may represents a potentially important risk factor for Parkinson's disease. To test this hypothesis, we propose to study the relationship between the genotype of the mutations and the phenotype of the disease, and to investigate the underlying mechanisms. First (Aim 1), we will assay for the mutations, genotypes, and phenotypes in the first-degree members of all 10 fPD patients with the identified NURR1 mutations to determine the haplotype linkage, relationship between genotype and phenotype. Second (Aim 2), we will investigate if the mutations in NURR1 gene are specific for Parkinson's disease by analyzing the gene mutations in a total of 400 NC, 300 sPD, and 120 non-PD neurological disorders. Finally (Aim 3), we will determine the mechanisms by which the mutant genes alter NURR1 expression and DAergic neuronal

Principal Investigator: LEE, MICHAEL K.
Grant Number: 1R21NS049088-01
Title: Conditional Uch-L1 knockout mice

Abstract: Ubiquitination is a post-translational modification of proteins that regulate a host of important cellular processes including degradation of cellular proteins, expression of genes, and protein/membrane trafficking. Ubiquitination of proteins and the specificity of ubiquitination are mediated by three classes of ubiquitin ligases (E1, E2, and E3). In addition to the ligases, a variety of deubiquitinating enzymes (DUBs) may regulate ubiquitination in cells. DUBs include ubiquitin carboxy-terminal hydrolase L1 (Uch-L1), a protein selectively expressed in neurons and in sertoli cells of testis. Defects in Ubiquitination/proteasomal mechanisms are implicated in the pathogenesis of many neurodegenerative diseases including Alzheimer's disease and Parkinson's disease. In particular, pathogenic relationship between defects in ubiquitin/proteasomal pathway and degeneration of dopaminergic neurons are indicated by the fact that PD-associated mutations are identified in Parkin, an E3-ubiquitin ligase, and Uch-L1. In gad mutant mice, deletion of exon 7 and 8 of Uch-L1 gene leads to degeneration of neurons in the DRG and in the gracile nucleus and early lethality. However, because Uch-L1 is expressed at high levels in many neuronal populations, Uch-L1 function may be important in other neurons. Because of early lethality and general underlying movement defect in gad mice, the functional importance of Uch-L1 in a various neuronal population and as a function of aging can not be examined effectively. We propose generate a mice where the expression of Uch-L1 can be temporally and spatially regulated. Specifically, we will generate UchL1-floxed mice to conditionally silence Uch-L1 expression by mating to appropriate Cre expressing mice. As an initial test of our hypothesis, we will determine whether Uch-L1 activity is important for normal functioning and aging of the dopaminergic and noradrenergic neurons by mating. The loxP-targeted Uch1 mice will be mated to pTH-Cre transgenic mice to silence Uch-L1 expression only in the dopaminergic and noradrenergic neurons.-

Principal Investigator: LEE, STEPHEN L
Grant Number: 5K08NS044298-02
Title: Genetic Analysis of Parkinsonism in an Ohio Amish Family

Abstract: Disease genes discovered through linkage analysis in familial Parkinson disease (PD) are yielding new insights into the pathogenesis of this neurodegenerative disorder. However, the known genes explain only a minor portion of all PD, and the chromosomal regions linked to other families are large and contain numerous genes. The discovery of additional hereditary causes of PD may help further elucidate the underlying etiopathogenesis and provide new pharmacological targets. It is therefore crucial that additional families are characterized. In an extended Amish family in northeastern Ohio, clinical information for familial Parkinsonism has been obtained. To test the hypothesis that genetic influences contribute to the expression of Parkinsonism in this Amish pedigree, the immediate aims of this project are three-fold: 1) to fully ascertain the disease phenotype of the affected individuals, through genealogical data, clinical history, medical records, and neurological exam, 2) to identify the genetic locus or loci associated with the disease phenotype, initially by evaluating previously identified genetic loci, and conducting a genome-wide scan using conventional linkage analysis, transcript mapping, and gene identification, and 3) to perform candidate gone analysis to test whether specific gene modifiers enhance or suppress the expression of the disease phenotype. The candidate's long-term goals are to apply well-established and emerging methods toward understanding the genetic basis of Parkinsonism. This grant will help the candidate establish an independent career in academic neurology with specialization in movement disorders and neurogenetics by allowing the candidate 1) to evaluate and treat patients in a movement disorders clinic under the guidance of Thomas L. Davis, M.D., and 2) to learn the laboratory, statistical, and computational methods of genetic epidemiology. This will be accomplished through conduct of the proposed research project and participation in formal courses under the guidance of the candidate's mentor, Jonathan L. Haines, Ph.D.-

Principal Investigator: LI, SENLIN
Grant Number: 1R01NS046004-01A1
Title: Macrophage Gene Therapy of Neurodegenerative Diseases

Abstract: Neurodegenerative diseases affect a large population of patients. Existing therapies are not satisfactory. Gene therapy holds promise, but focal delivery of DNA and the level of gene expression are challenging. Macrophages are recruited from bone marrow to most tissues of the body including the CNS, thus making them an attractive option for gene delivery. Galactosialidosis (GS) has been corrected by bone marrow-derived macrophages expressing human protective protein/cathepsin A (PPCA) transgene in a mouse model (PPCA-/-). However, correction in the CNS was incomplete due in part to weakness of the CSF-1R promoter used in the study. We have developed a series of super macrophage promoters (SMP) that are up to l00-fold stronger in vitro than the CSF-1R promoter. In models of the highly prevalent Parkinson's disease (PD), local delivery of glial cell line-derived neurotrophic factor (GDNF) has been found beneficial. We hypothesize that highly effective CNS delivery of GDNF can be achieved with the use of our super macrophage promoters and this will greatly ameliorate the pathologic changes and neurological defects in animal models of PD. To explore this hypothesis, our specific aims are: 1) To characterize these super macrophage promoters by transplantation of bone marrow stem cells transduced ex vivo with lentiviral vectors and in transgenic mice using EGFP (enhanced green fluorescent protein) as a reporter. Promoters with the greatest strength and tissue-specificity for macrophages will be used in the subsequent aims. 2) To ameliorate neurodegeneration in the MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) mouse model of Parkinson's disease by syngeneic transplantation of HSC transduced ex vivo with lentivectors expressing GDNF gene in macrophages/macroglia driven by the SMP. Bone marrow stem cells will be transduced ex vivo with GDNF expressing lentivirus and transplanted into lethally irradiated recipient mice. Four weeks after bone marrow transplantation, the recipient mice will be injected subcutaneously with MPTP. At selected time points post MPTP administration, PET scan and behavioral testing will be performed, and brain tissue will be examined for dopamine uptake and expression of tyrosine hydroxylase (TH). In the substantia nigra pars compacta (SN), dopaminergic neurons will be counted and cell apoptosis will be assessed by TUNEL staining and immunohistochemistry for active easpase-3. 3) To ameliorate neurodegeneration in the same way as in Aim 2, but GDNF expression will be controlled by a tetracycline-regulatable gene expression system. To evaluate the effects of macrophage/ super promoter-mediated delivery and expression of GDNF on degenerating

Principal Investigator: LIU, YICHIN
Grant Number: 5F32NS042415-03
Title: Ubiquitylation of alpha-synuclein by UCH-L1

Abstract: Parkinson?s disease (PD) is characterized by the presence of Lewy bodies, the cytoplasmic neuronal inclusions, in the substantia nigra. One major component of the Lewy bodies was identified to be the fibril a-synuclein, thus linked the accumulation of this protein to the pathogenesis of PD. Failure to regulate the concentration of a-synuclein can contribute to the build-up and consequently fibrillization of the protein. The objective of this proposed research is to investigate the degradation process of a-synuclein through the ubiquitin/proteasome pathway. Neuronal ubiquitin C-terminal hydrolase (UCH-L1) hydrolyzes C-terminal ester and amides of ubiquitin and is believed to play a key role in processing polyubiquitin and/or ubiquitylated proteolytic peptide. A mutation (I93M) of the enzyme has been linked to a rare early-onset form of PD, at the same time a polymorphism of the enzyme (S18Y) was indicated to reduce the risk of PD. By direct comparing the specific activities of the wild-type and mutant UCH-L1 enzymes using techniques in biochemistry, biophysics and molecular biology, we hope we will have a better understanding of the degradation pathway of a-synuclein and the role of the UCH-L1 in the proteasome.-

Principal Investigator: MARDER, KAREN S
Grant Number: 2R01NS036630-05A1
Title: Genetic Epidemiology of Parkinson's Disease

Abstract: In the first funding period, we compared the risk of PD in relatives of 221 PD patients with age at onset (AAO) < 50 to relatives of 266 PD patients with AAO >50 and 409 controls. The magnitude of increased risk of relatives of PD cases vs. controls was similar in early-onset cases (RR: 2.9, 95%C1: 1.6-5.0) and late onset cases (RR: 2.7, 95%CI: 1.6-4.4). However in families of early-onset cases, the degree of increased risk was much greater in siblings (RR: 7.9, 95%C1: 2.5-25.5) than in parents (RR: 1.7, 95% CI: 0.9-3.3), consistent with an autosomal recessive contribution to inheritance. In late-onset families, risk was elevated in both parents and siblings, inconsistent with a recessive model. Mutations in the parkin gene have emerged as the most important causative or risk-raising factor in early-onset PD. In this second competitive renewal application, we have redesigned our study to make optimal use of 300 early onset cases already recruited at the Columbia site (200 not yet screened for parkin mutations) and the 40 PD cases with parkin mutations we have already identified. We will join with investigators at 7 other sites who will contribute an additional 600 cases with age of onset < 50 and 21 identified parkin families to form a US Parkin Consortium. Our first aim is the expansion of 125 PD cases that carry parkin mutations to include 1st and 2nd degree relatives. We will determine whether the risk of psychiatric and cognitive manifestations in asymptomatic gene carriers who do not meet criteria for PD is higher than in asymptomatic non-gene carriers. Identification of a parkin carrier phenotype will provide clues to etiopathogenesis and may define the appropriate time for early therapeutic intervention. Our second aim is to define the distribution of age specific penetrance in 100 of the 125 families who were recruited solely be age of onset, so as not to bias these estimates upward by inclusion of "high risk" families recruited because they are multiplex. We will compare differences in age specific penetrance by allelotype (heterozygous vs. homozygous or compound heterozygous). The results of this study will clarify the role of parkin in genetic susceptibility and foster the development of genetic testing guidelines. The consistent finding that at least 30 percent of PD patients with parkin mutations are heterozygotes, despite that fact that inheritance was initially described as recessive, and new availability of commercial testing make this study both critical and timely. -

Principal Investigator: MATTERN, MICHAEL R
Grant Number: 5R43NS047948-02
Title: Screen For Inhibitors of Parkin E3 Autoubiquition

Abstract: Neurodegenerative disease represents a major challenge to the maintenance of health and quality of life in diverse segments of the population. Among the various diseases of this class, Parkinson's disease (PD) is a major cause of morbidity and diminished life expectancy, and there is today an intense effort to discover novel treatments. A promising approach for therapeutic intervention is treatment designed to increase the cellular level of parkin, a protein which has been found to antagonize neurodegeneration in model systems, and which is linked genetically with some forms of PD. The selection and validation of targets that can be manipulated to achieve this effect depends on an increasing amount of information relating to parkin regulation. A novel area for drug discovery -- protein homeostatic regulation via ubiquitin pathway enzymes -- has recently been demonstrated to have relevance to the search for anti-neurodegenerative drugs. Parkin has, in fact, been determined to be a RING-finger E3 ubiquitin ligase that catalyses ubiquitination and, subsequently, induces proteasomal degradation of various proteins associated with neurodegeneration. In addition, it catalyses its own ubiquitination. Thus, selective inhibitors of parkin autoubiquitination are hypothesized to have a neuroprotective effect. In Phase I, it is proposed to establish a yeast-based screening assay for inhibitors of parkin autoubiquitination and a selectivity counter screen for ubiquitination of alpha-synuclein, a parkin substrate. Essential components of the E3 system (parkin) will be cloned and expressed in S. cerevisiae, along with human parkin or alpha-synuclein linked to p53, and a reporter construct that monitors p53 activity (beta-galactosidase activation). The reconstructed E3 ligase function and reporter system will then be configured and validated as a high throughput screen for inhibitors of parkin autoubiquitination. Collections of plant and marine organism extracts and small molecules from the NCI and academic collaborators, will be screened for potent inhibitors of this activity. In Phase II, fractionation of active extracts will be guided by the assay to identify active principles. Novel pure compounds arising from this effort will be considered as development candidates for PD therapy. The modular assay construction format will permit evaluation of other E3s that are associated with a variety of diseases.-

Principal Investigator: MCDONALD, PAUL W
Grant Number: 5F31NS046237-02
Title: Using C. elegans to Investigate the Dopamine Transporter

Abstract: The dopamine (DA) transporter (DAT) is critical in the re-uptake of DA into presynaptic neurons and the termination of dopamine signaling. Alterations in DA signaling is evident in several disease pathologies including ADHD, Parkinson's disease and schizophrenia. Recently, it has been shown that intracellular proteins interact with and affect the localization and function of DAT. As such, we hypothesize that DAT exists in a protein complex that regulates the activity of the transporter. To test this hypothesis we will take advantage of the model system C. elegans to test candidate regulatory proteins for DAT and identify novel proteins that associate with the transporter. The C. elegans dopamine transporter DAT-1) shows a 45% homology to the human transporter, with a sensitivity to amphetamines, cocaine, and other biogenic amine transporter antagonists. In my proposal, I build on recent studies by our laboratory on DAT-1 to: 1) determine the expression level and localization of DAT-1, 2) characterize the interactions of the C. elegans PICK1 homologue (Y57G11C.22) with DAT-1 and, 3) identify novel proteins that interact with the C. elegans dopamine transporter. These studies will increase our understanding of intrinsic modulatory influences controlling DA signaling in vivo and in disease states.-

Principal Investigator: MCNAUGHT, KEVIN S
Grant Number: 1R01NS045999-01A1
Title: ROLE OF PROTEASOMAL DYSFUNCTION IN PARKINSON'S DISEASE

Abstract: Parkinson's disease is characterized pathologically by selective degeneration of dopamine-containing neurons in the substantia nigra pars compacta (SNc). The etiology in the vast majority of individuals with the disorder remains elusive but ageing is an important risk factor. Nigral cell death in PD is accompanied by the accumulation of oxidatively damaged proteins, aggregation of proteins and the formation of proteinaceous intracytoplasmic Lewy body inclusions. These observations suggest that failure of the ubiquitin-proteasome system (UPS), the biochemical pathway primarily responsible for the degradation of abnormal and short-lived regulatory/transcriptional proteins may underlie nigral pathology in Parkinson's disease. Indeed, mutations in the genes encoding alpha-synuclein and 2 enzymes of the UPS, namely parkin and ubiquitin Cterminal hydrolase L1, are associated with altered protein handling in rare familial forms of Parkinson's disease. However, these or similar gene defects do not occur in most patients who have sporadic Parkinson's disease. We hypothesize that defects in 26/20S proteasomes cause the UPS to fail and this underlies protein accumulation, Lewy body formation and dopaminergic neuronal death in the SNc in sporadic Parkinson's disease. Consistent with this hypothesis, our preliminary findings demonstrated structural and function defects in 26/20S proteasomes, and a several-fold increase in the levels of poorly degraded/undegraded and potentially cytotoxic ubiquitinated protein substrates, in the SNc but not elsewhere in sporadic Parkinson's disease. In addition, we showed that in aged control subjects and adult rats, dopaminergic neurons of the SNc have relatively low 26/20S proteasomal activity and poor expression of the proteasome activators (PA28 and PA700) compared to other brain regions. Further, we showed that inhibition of 26/20S proteasomal function causes selective degeneration of dopaminergic neurons with the formation of alpha-synuclein/ubiquitin-immunoreactive inclusions in primary mesencephalic cultures and in rat SNc with motor dysfunction. In this project, we propose to determine (1) if and how the structure and function of proteasomes are defective in all stages of sporadic PD; (2) if low proteasomal function normally occurs in the SNc of controls as this may underlie its selective vulnerability and degeneration in PD; (3) if proteasomal dysfunction underlies Lewy body formation; and (4) if proteasomal dysfunction plays a role in nigral dopaminergic cell death in sporadic Parkinson's disease. These studies will test our hypothesis that inadequate proteasomal function underlies both vulnerability and degeneration of the SNc in sporadic Parkinson's disease.-

Principal Investigator: Meredith, Gloria
Grant Number: 5R01NS041799-05
Title: Synaptic Proteins, Trophic Factors and Neurodegeneration

Abstract: One of the most fundamental questions related to the progressive nature of neurodegeneration in human disease is how neurons die. Protecting nerve cells against morphological decline and death requires blocking intrinsic factors that inhibit neural repair. In the present proposal, we offer an innovative approach to study those factors that are active in Parkinson's disease (PD) in a new mouse model that shows synaptic loss and irreversible nigrostriatal degeneration. We propose to track changes of a key synaptic protein, a-synuclein, both in its native environment at presynaptic terminals and under neurotoxic conditions, when it becomes insoluble and accumulates. We will further correlate those changes with altered neurotrophic support. We have established an animal protocol by treating C57/bl mice with a combined regimen of 10 doses of probenecid at 250mg/kg and MPTP at 25mg/kg for 5 weeks. These mice show a slow, progressive loss of nigrostriatal dopaminergic function for at least 6 months, that mimics PD, with no signs of recovery. Three weeks after drug treatment, there is a significant reduction in the number of substantia nigra (SN) cells and dramatic changes in the subsynaptic distribution and density of a-synuclein-immunoreactive terminals. These changes could signal the beginning of a chain of events that leads to cell death. In this proposal, we will focus on the progressive deterioration of dopaminergic neurons in the SN and their inputs, and present three specific aims to be addressed through a series of hypotheses. Specifically, we plan to 1) ascertain the origin and neurochemical phenotype of synapses in the SN that contain a-synuclein and to establish whether MPTP + probenecid treatment leads to their degeneration; 2) determine, in the MPTP+P model, the temporal relationships between cell death and a-synuclein-positive synapses, decline in dopamine function and behavior; and 3) ascertain whether changes in a-synuclein expression and production are precipitated by altered neurotrophic support. The overall objective of our research is to understand the relationship between the synaptic protein, a-synuclein, neurotrophic support, especially brain-derived neurotrophic factor (BDNF) and their respective roles in the PD form of neurodegeneration. The findings of this research should shed light on target areas where neuroprotection strategies can be implemented. -

Principal Investigator: MOSLEY, RODNEY L
Grant Number: 1R21NS049264-01
Title: Neuroprotective Vaccination for Parkinson's Disease

Abstract: Microglia inflammation contributes, in significant measure, to the loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) during idiopathic Parkinson's disease (PD). Attenuation of such inflammation could attenuate disease. To this end we show that microglial deactivation responses, induced by vaccination, in 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) intoxicated mice improves dopaminergic neuronal survival. This was achieved by adoptively transferring spleen cells from copolymer-1 (Cop-1) immunized mice to MPTP-treated recipients. Spleen cells from ovalbumin (OVA) injected mice failed to affect neuronal protection. Thus, our preliminary works show that protection from dopaminergic neurodegeneration can be achieved by adaptive immunity with T cells specific for Cop-1. Based on response kinetics, antigen specificity, and functional adaptive T cell immune responses, we predict that the mechanism(s) of neuroprotective immunity can be realized and could provide novel treatment strategies for human disease. Our hypothesis posits that protection from dopaminergic neurodegeneration by Cop-1 vaccination is generated through immune cell-mediated mechanisms with specificity for Cop-1 peptides and self-antigens. To investigate this we will adoptively transfer T lymphocytes, B cells and monocytes from Cop-1 immunized mice into MPTP-treated animals. Neuroprotection will be assessed by numbers of dopaminergic neurons, neurotransmitter levels, and neuronal metabolites by magnetic resonance spectroscopic imaging (MRSI). Immune cell populations, proven relevant to neuroprotection will be evaluated for the expression of gene products that are cell population specific as candidates for neuroprotection. Genetic fingerprint analysis will include cDNA microarray analysis and proteomics. This approach takes advantage of an integrated and well-established research program within the Center for Neurovirology and Neurodegenerative Disorders and builds upon research activities in PD supported previously through private donations. These approaches could prove useful for treatment of human PD. -

Principal Investigator: MUCHOWSKI, PAUL J
Grant Number: 1R01NS047237-01
Title: Modifiers of Huntingtin and Alpha-synuclein Toxicity

Abstract: Huntington's disease (HD) is an autosomal dominant inherited disorder characterized by involuntary movements, personality changes and dementia, and is caused by an expansion of a CAG/polyglutamine repeat in the IT-15 gene. A major neuropathological hallmark in HD is the occurrence of intranuclear and cytoplasmic inclusion bodies that contain huntingtin (the protein encoded by IT-15). Cytoplasmic inclusion bodies (Lewy bodies) are also a prominent nature of Parkinson's disease (PD), a neurodegenerative disorder characterized by muscle rigidity, bradykinesia, resting tremor and postural instability. Lewy bodies are composed primarily of the protein alpha-synuclein, and two point mutations in the alpha-synuclein gene cause early-onset, inherited forms of Parkinson's disease. Alpha-synuclein and huntingtin aggregate into ordered fibrillar structures with properties characteristic of amyloid. The 'amyloid hypothesis', developed originally to describe the role of beta-amyloid in Alzheimer's Disease (AD), suggests that the aggregation of proteins into an ordered fibrillar structure is causally related to aberrant protein interactions that culminate in neuronal dysfunction and cell death (Hardy and Selkoe, 2002). The precise roles of protein aggregation, amyloid formation and inclusion bodies in neurodegeneration remain controversial, and it is not yet clear if common molecular mechanisms underlie HD and Parkinson's disease. We have used yeast as a model eukaryotic organism to test the hypothesis that the downstream targets and molecular mechanisms by which huntingtin and ot-synuclein mediate toxicity are unique. Using a genome-wide screening approach in yeast we isolated 52 genes that modify huntingtin toxicity, and 86 genes that modify alpha-synuclein toxicity. 30% of genes that affect huntingtin toxicity are enriched in the functionally related categories of protein folding and cell stress, while 29% of genes that modify alpha-synuclein toxicity are involved in vesicular transport and lipid metabolism. Our preliminary results indicate surprisingly that the genes and cellular pathways that modulate huntingtin and alpha-synuclein toxicity in yeast are completely divergent. Nearly half of the genes we isolated are annotated as having one or more human ortholog, suggesting we may have discovered in yeast conserved cell-biological response pathways to huntingtin and alpha-synuclein that are relevant to HD and Parkinson's disease. Using the resources and information that we have generated, we now wish to advance our understanding of the neurodegeneration that occurs in HD and PD by applying molecular genetic and biochemical techniques to validate (or invalidate) the genetic modifiers we have identified. Our

Principal Investigator: MURCIA, CRYSTAL L
Grant Number: 5F32NS043844-03
Title: The Role of the En-I Pathway in Parkinson's Disease

Abstract: Parkinson?s disease (PD) is a common progressive neurodegenerative disease affecting approximately 2 percent of the population over age 65 throughout the world. Evidence from studies of idiopathic PD suggests that it is a complex disease involving multiple genetic and environmental factors. We have developed a potential mouse model of idiopathic Parkinson?s disease in the Engrailed-i (En-i) knockout mouse, En aboutiM. Homozygous En about ih/d mice on the 129/Sv inbred strain display severe cerebellar hypoplasia and perinatal lethality. In contrast, on the C57B1/6J inbred strain, homozygous mice are viable and exhibit tremors and hesitant gate reminiscent of Parkinson?s disease. To further analyze the role of strain background on the En-i mutant phenotype we propose the following three specific aims: 1) further characterize the Parkinson?s disease phenotype of En-i deficient mice, 2) genetically map strain-specific modifier genes required to produce the PD phenotype in En-1hd mutant mice, and 3) analyze candidate modifier genes for strain-specific alterations that contribute to the PD phenotype. The discovery of new genes with a genetic link to PD will provide future targets for therapeutic intervention.-

Principal Investigator: MUZYCZKA, NICHOLAS
Grant Number: 3P01NS036302-06A1S1
Title: Adeno-Associated Virus Gene Transfer to Nervous System

Abstract: Unavailable

Principal Investigator: MUZYCZKA, NICHOLAS
Grant Number: 2P01NS036302-06A1
Title: Adeno-Associated Virus Gene Transfer to Nervous System

Abstract: The long term goal of this Program is to develop gene transfer methods for the treatment of neural disorders. Three groups that are well integrated have come together to develop methods for using recombinant Adeno-associated virus (rAAV) for the treatment of retinal and CNS neurodegenerative diseases. Project 1 (Muzyczka) proposes genetic experiments to identify the proteins in the substantia nigra and striatum that interact with alpha synuclein. It will specifically examine alpha syn interactions with GRK and PLD2, and develop for the first time somatic knockouts of GRK and PLD2 using AAV vectors. It will also examine the effect of oxidative stress in combination with alpha syn overexpression on neurodegeneration in the substantia nigra. Finally, it will use biochemical techniques to directly identify protein complexes that contain alpha syn. Project 2 (Hauswirth and Lewin) will take the next step toward developing a therapy for P23H rhodopsin RP using the ribozymes they developed in the previous grant period. Further, they will test two new strategies for RP that are likely to be of more general use for all RP diseases. The first is the use of GDNF expression to promote photoreceptor survival. The second is to replace all (wild type and mutant) endogenous rhodopsin mRNAs with a wild type mRNA. If successful, this should prove to be a general approach that could be applied to all genetic RP, regardless of the point mutant involved. Project 4 (Mandel) will extend their preclinical experiments toward developing AAV mediated gene transfer for Parkinson disease. Specifically, they will develop regulatable GDNF constructs that are a prerequisite for clinical applications, do the first comprehensive analysis of the immune response to AAV vectors that are injected into the brain, and test their therapeutic GNDF strategy in a primate model of Parkinson's to obtain dosing information and confirmation of efficacy in a brain model closer to human. Two cores are also proposed. Core A (Administration) will continue in its role of providing fiscal/administrative support, educational programs, and program oversight in the form of internal and external advisors. The Vector Core will continue to improve the efficiency and scaleability of rAAV vectors. In addition to providing the routine service of production and purification of rAAV2-based vectors, the Core will also develop methods for purification of alternative AAV serotypes and capsid mutants to be used in projects 1, 2, and 3.-

Principal Investigator: MYERS, RICHARD H
Grant Number: 5R01NS036711-07
Title: Genetic Linkage Study in Parkinson's Disease

Abstract: The cause of idiopathic Parkinson's disease (PD), a debilitating disease that afflicts an estimated 1 percent of persons age 60 or older, remains unknown. In this application, we propose genetic epidemiological studies, genetic linkage studies, and candidate gene studies for PD. In the past four years, we have established a ulti-institutional research program that has collected 300 PD affected sibling pairs and extended families. Our linkage analyses to onset age revealed evidence for linkage (LOD 2.1) to chromosome 2pl3 at the 'PARK3' locus (Gasser et al. 1998). Recently, we found association (p< 0.02) to the same STR marker and allele as seen by Dr. Gasser's group and association to SNP alleles (pC0.008) We also see evidence for linkage to two other loci reported by other groups. Our reported linkage to PD affection on chromosome 9q (DeStefano et al. 2001) is also reported by Scott et al. (2001). Drs. Hardy and Farrer of the Mayo Clinic Jacksonville confirm linkage to chromosome 10q in the same region that we reported for PD affection. Thus, we have detected at least three regions (2p, 9q, and 10q) that harbor PD related genes detected by other groups. These findings confirm that PD is a complex trait, requiring a large well-characterized sample for sufficient power to identify the implicated genes. We propose (AIM 1) genetic epidemiological studies aimed at identifying factors related to penetrance in PD by studying risk factors predicting onset age in sibling pairs who are widely discordant for onset age. This unique sample of sibling pairs permits novel analyses of factors related to penetrance. We propose (AIM 2) to continue our genetic linkage analysis, with a 10cM density genome scan in 350 affected sibling pairs and other family members; 300 of these affected sibling pairs have already been collected. We will assess possible genetic heterogeneity associated with risk factor involvement and PD family history. We have found significant modification of onset age. We propose (AIM 4) to follow-up those regions with evidence for linkage to PD affection or onset age in and additional 350 PD sib pairs to be collected in this study. We further propose association studies to localize candidate genes. Finally, we propose (AIM 5) a focused candidate gene study for PD, concentrating primarily on genes and genomic regions implicated in dystonia, due to overlapping clinical features of PD and dystonia. This project has great potential to expand our knowledge of the genetics of PD and to identify PD associated genes and risk factors and patterns for their interaction. -

Principal Investigator: NELSON, LORENE M
Grant Number: 5R01NS031964-08
Title: Environmental and Genetic Risks for Parkinson's Disease

Abstract: The primary goal of this project is to investigate whether certain environmental exposures and genetic variants, either alone or in combination, affect the risk of developing Parkinson's disease (PD). We will investigate mechanisms that may explain the consistently observed inverse associations of cigarette smoking and caffeine consumption with PD, and the role of residential pesticide exposure on the risk of PD. In addition, existing and newly-identified polymorphisms in the coding and regulatory regions of candidate genes will be investigated, including genes that code for: (1) endogenous enzymes involved in metabolism of tobacco or caffeine, or in the detoxification of putative toxicants for PD, (2) proteins involved in dopamine regulation or metabolism, and (3) proteins that play a role in protein degradation and aggregation in dopaminergic neurons. We propose to expand a recently completed case-control study in a large health maintenance population of more than 500 newly diagnosed PD cases and 500 controls. Because preliminary data show that the strongest associations of genetic variants were observed among PD cases with early age at diagnosis (age less than or equal to 60), we will identify approximately 330 additional such cases along with age- and sex-matched controls. This new sample will be combined with that of the previous study, resulting in approximately 420 young diagnosis cases and 430 older diagnosis (age greater than 60) cases to be compared with 870 age- and gender-matched controls. Detailed information will be collected from all study subjects using in- person and telephone structured interviews including information on cigarette smoking, caffeine intake, and residential exposure to pesticides, along with other putative risk factors. Venous blood samples will be drawn for DNA extraction and genotyping assays for the gene polymorphisms of interest. By examining genetic polymorphisms within a group of carefully chosen candidate genes, in combination with extensive information about common environmental exposures, we hope to advance knowledge regarding both genetic and modifiable environmental risk factors for Parkinson's disease. -

Principal Investigator: O'MALLEY, KAREN L
Grant Number: 2R01NS039084-05A1
Title: Mechanisms of Neuronal Death in Parkinson's Disease

Abstract: Oxidative stress is a major factor in Parkinson's Disease (PD). Dopamine (DA) itself is easily oxidized to quinone derivatives and reactive oxygen species (ROS) that impair energy metabolism and form adducts with proteins such as upsilon-synuclein. Because pharmacological depletion of DA in animal models is confounded by non-specific peripheral and central nervous system effects, the role of DA oxidation in nigral cell death has been previously impossible to address. Thus a key unanswered hypothesis in this field is that DA oxidation is a major contributor to the death of dopaminergic neurons in PD. The proposed studies address several aspects of this hypothesis including the interaction of known environmental factors in triggering DA oxidation. Specifically, the hypothesis that the DA-releasing potential of the parkinsonism-inducing drug, MPP+, is due to its ability to exchange with DA and/or to reduce intracellular pH gradients will be addressed using newly derived mice expressing enhanced green fluorescent protein from a dopaminergic locus (TH+/eGFP). Primary cultures derived from these animals as well purified synaptosomal and vesicular preparations from dopaminergic terminal fields will be used in combination with fluorescent and radioactive probes to determine the temporal aspects of DA release, intracellular membrane changes, ROS formation, ATP loss, etc in response to toxin treatment. In addition, the hypothesis that DA oxidation contributes to the death of dopaminergic cells will be directly tested in vivo using animals genetically engineered to have different levels of DA production. Behavioral, oxidative and immunocytochemical criteria will be used to establish the role of DA in both the acute and chronic MPTP model of PD. To test whether DA depletion prevents ROS, new methodologies to detect in situ ROS will be used with a battery of antibodies directed against nitrotyrosine, nitrated alpha-synuclein, etc. to temporally evaluate ROS formation following acute or chronic MPTP administration in DA deficient and wild type animals. Taken together, the proposed studies will determine whether DA oxidation plays a central role in the death of DA synthesizing cells and provide insights impossible to obtain from standard animal models. Knowledge of the source and cascade of events surrounding DA-induced free radical formation will help answer risk-benefit controversies surrounding the use of dopamine replacement therapies as well as facilitate the development of new drugs and/or treatment strategies in the pathogenesis of PD. -

Principal Investigator: PALLANCK, LEO J
Grant Number: 5R01NS041780-04
Title: Parkin mediated Neural Dysfunction in Drosophila

Abstract: Parkinson's disease is a prevalent neurodegenerative disorder characterized by tremors, rigidity, and bradykinesia. These symptoms are thought to arise from the degeneration of dopaminergic neurons in the substantia nigra pars compacta. Recently, mutations of the parkin gene, which encodes a ubiquitin-protein ligase, were found to underlie a familial form of Parkinson's disease known as autosomal recessive juvenile Parkinson's disease (AR-JP). While this advance provides clues to the mechanism responsible for pathology in AR-JP, the cellular targets of the parkin ubiquitin-protein ligase activity and the specific biochemical pathways affected by parkin mutations remain largely unknown. To address these issues, the objectives of this proposal are to create a Drosophila melanogaster model of AR-JP through mutational analysis of a Drosophila parkin ortholog, and to use this fly AR-JP model to investigate the molecular mechanisms of neuronal dysfunction underlying parkin deficiency. Two main hypotheses will be explored in this proposal: (a) parkin sequesters alpha-synuclein protein into Lewy bodies and this function represents a cellular mechanism of alpha-synuclein detoxification; (b) neurodegeneration triggered by parkin mutations results from accumulation of parkin substrate(s). To accomplish the objectives of this proposal, the following specific aims will be pursued: (1) Generate and characterize Drosophila parkin (D-parkin) mutants; (2) Determine whether altered D-parkin expression affects the time course and extent of alpha-synuclein induced neurodegeneration and Lewy body formation; (3) Identify modifiers of a D-parkin mutant phenotype; (4) Isolate D-parkin-binding components and investigate structure-function relationships in D-parkin. Results from this work should clarify the relationship between parkin dysfunction and neurodegeneration, and possibly reveal strategies for treatment of Parkinson's disease. -

Principal Investigator: PALLANCK, LEO J
Grant Number: 1R21NS048362-01
Title: Mutational Analyses of Drosophila DJ-1 Homologs

Abstract: Parkinson's disease is a prevalent neurodegenerative disorder characterized by tremors, rigidity, and bradykinesia. These symptoms arise from the degeneration of dopaminergic neurons in the substantia nigra. The cellular and molecular mechanisms responsible for neurodegeneration in Parkinson's disease remain poorly understood, although genetic and environmental factors both appear to play contributing roles. Recently, loss-of-function mutations in DJ-1, a gene of unknown function, were found to be responsible for an autosomal recessive form of Parkinson's disease. To explore the normal biological function of DJ-1, and the mechanism by which loss of DJ-1 function results in neurodegeneration, we propose to subject a pair of highly conserved Drosophila DJ-1 homologs (designated DJ-1a and DJ-1b) to mutational analysis. DJ-1a and DJ-1b function will be perturbed using P element mutagenesis, gene-targeting and double stranded RNA interference methods. The phenotypes resulting from perturbation of these genes will be fully characterized, including an analysis of dopaminergic neuron integrity. Additionally, we will characterize the global gene expression changes resulting from loss of DJ-1a and DJ-1b function and initiate screens for genetic modifiers of the DJ-1a and DJ-1b phenotypes to elucidate the biochemical pathways in which these genes function. This work should clarify the normal cellular role of DJ-1 and provide a foundation for further hypothesis-driven investigation of DJ-1 function. -

Principal Investigator: PATEL, MANISHA
Grant Number: 1R01NS045748-01A1
Title: Mitochondrial Aconitase and Parkinson's Disease

Abstract: The long-term goal of this proposal is to elucidate the mechanism by which mitochondrial oxidative stress produces dopaminergic neuronal death in Parkinson's Disease (PD). The precise mechanism by which mitochondrial oxidative stress, bioenergetic decline and iron overload arise and collaborate to produce age-related neuronal death in Parkinson's disease remains unclear. It is hypothesized that neuronal damage in Parkinson's disease results, in part from direct superoxide radical toxicity due to oxidative inactivation of mitochondrial aconitase. The hypothesis predicts that superoxide production, arising from Complex I inhibition or abnormal dopamine metabolism, inactivates [4Fe-4S]Z+-containing mitochondrial aconitase, resulting in loss of aconitase activity and release Fe z+ and H202. Posttranslational modification of this key TCA cycle enzyme can therefore result in an increased iron load, oxidant burden and bioenergetic decline. The presence of an iron responsive element (IRE) in the 5' untranslated region of the mitochondrial aconitase Mrna provides an additional mechanism for iron dysregulation in Parkinson's disease. The proposal will utilize human PD samples, animal models of PD (1-methyl-4-phenyl-l,2,3,6-tetrahydropyridine and 6-hydroxydopamine) and dopaminergic cell culture models in conjunction with a diversity of tools and techniques that include biochemical analyses, confocal microscopy, molecular biology and transgenic/knockout/aging mice. Specific Aim 1 will determine whether mitochondrial aconitase is inactivated in human and experimental Parkinson's disease. The influence of aging and chronic mitochondrial oxidative stress will be determined using mice deficient in MnSOD, a critical mitochondrial antioxidant. Specific Aim 2 will determine whether mitochondrial aconitase inactivation contributes to impaired iron homeostasis. Specific Aim 3 will determine whether scavenging mitochondrial superoxide using native or synthetic antioxidants (e.g. MnSOD transgenic mice or metalloporphyrins) protect against mitochondrial aconitase inactivation in a manner that correlates with decreased iron overload and neuronal death in experimental Parkinson's disease. Specific Aim 4 will determine the downstream consequences of mitochondrial aconitase inactivation in experimental Parkinson's disease. Specifically, regulation of brain mitochondrial aconitase synthesis by the 5' IRE in its mRNA, impact on the TCA cycle capacity and direct neurotoxicity of aconitase gene silencing will be examined. These studies can advance our understanding of the oxidative mechanisms of neuronal death in Parkinson's disease and suggest novel therapeutic strategies

Principal Investigator: PAYAMI, HAYDEH
Grant Number: 5R01NS036960-07
Title: Genetic Analysis of Onset Age of Parkinson's Disease

Abstract: The goal is to avoid Parkinson's disease (PD) either by eliminating the cause or delaying the onset. PD is heterogeneous, with a significant genetic component. The working hypothesis is that some genes such as asynuclein and parkin cause PD, others determine ones' susceptibility to neurotoxins and injury, and a third set modulate disease progression and age at onset. Originally studied in the rare autosomal recessive juvenile PD, recent studies suggest parkin may also be involved in common forms of PD. We propose to study parkin in 609 PD patients, 609 control subjects, 100 newborns, 100 healthy elderly, and the key relatives of patients. The specific aims are to: (1) Determine the frequency of parkin mutations in PD. The impact of parkin in common PD is unknown. We will divide the patients into two groups, each with 300 subjects. The first group will be sequenced and analyzed for deletions and multiplications, to identify new and known mutations. The second group will be screened for mutations found in the first group. (2) Determine age-specific frequency of parkin mutations in controls. The frequency of parkin variations in the population is unknown. We will divide the controls into two groups, each with 300 subjects. The first group will be sequenced and analyzed for deletions and multiplications, to identify new and known mutations, including those that may be absent in patients (protective). The second group will be screened for mutations found in the first group. To examine allele frequency changes by age, 100 newborns and 100 healthy elderly will be studied. (3) Identify and distinguish disease-associated alleles, protective alleles, age at onset modifiers and neutral polymorphisms. We will analyze the first group of 300 patients and 300 controls, confirm the findings in the second group, and perform family-based tests of linkage disequilibrium to rule out false associations due to population stratification. (4) Study mode of inheritance, penetrance and age at onset of parkin mutations. Genotype-phenotype correlation will be used to determine mode of inheritance and test the hypothesis that parkin mutation dosage can cause anticipation. Understanding the mode of inheritance is essential for studies of disease pathogenesis and for counseling families about the risk. -

Principal Investigator: RACETTE, BRAD A
Grant Number: 5K23NS043351-03
Title: GENETICS OF PARKINSON DISEASE IN THE AMISH

Abstract: The applicant is a neurologist and movement disorders specialist with three years of post-fellowship, faculty experience involving clinical care, clinical trials, and clinical research into etiologic risk factors for PD including genetic factors. The goal of this career development award is to provide the applicant with comprehensive training in genetic epidemiology through course work, individual tutorials, and practical application of gene mapping techniques to a multi-incident Amish family with Parkinson Disease (PD). PD is a neurodegenerative disorder that produces substantial disability for nearly 1 million people in North America. There is no known cause of the disease in the majority of patients; however, a genetic etiology has been found in a few rare multi-incidence families. Identification of such genes and subsequent determination of the cell biological effects of these mutations will provide important clues to the pathophysiology. Each new mutation discovered adds critical converging evidence about pathophysiological mechanisms common to all to those affected with PD. We have identified 27 members of a large Amish family with clinically typical PD and have excluded known PD genetic mutations. However, we still need to prove that PD is inherited in this pedigree. We will use two different methods to prove that PD in this kindred has a genetic basis. The first approach will assume an autosomal recessive model of inheritance and use genetic marker data provided by CIDR on our subjects to perform homozygosity mapping. A second approach will be to calculate a kinship coefficient to determine if the affected members of the pedigree are "more related" than randomly selected age-matched individuals from the same population. Finally, we will test whether [18]FDOPA PET permits the conversion of some people identified clinically as possible or probable PD in to PET-confirmed PD and thereby functioning as an endophenotype for disease state. This family provides a unique opportunity for the candidate to become a productive independent investigator in genetics of Parkinson Disease and other movements disorders and to develop skills needed for interpretation of [18]FDOPA PET.-

Principal Investigator: REDMOND, D EUGENE
Grant Number: 1U01NS046028-01A1
Title: GDNF Delivery to MPTP Monkeys by EIAV lentivirus and AAV

Abstract: An effective gene therapy for Parkinson's disease is the goal of this proposal, which will test the effectiveness and safety of human glial cell line derived neurotrophic factor (GDNF) delivered by two improved vector systems derived from equine infectious anemia virus (EIAV) or from adenoassociated virus (AAV). Both vectors deliver the cellular marker gene, nuclear localized lacZ (lacZnl) or GDNF efficiently and stably into nigrostriatal target regions, can be regulated using a tetracycline promoter system, and offer additional safety that the respective wild-type viruses do not cause any disease in humans. The recombinant vectors will be tested in the parkinsonian model produced by the neurotoxin MPTP in monkeys. GDNF has shown promise for preventing or reversing morphological, biochemical and functional deficits in other models of Parkinson's disease in rodents and primates, using rAAV, and rHIV. But these studies also showed important problems to be solved to ensure that a GDNF gene therapy will be safe and effective in patients. Concerns about inflammatory, cytotoxic, inadequate or excessive gene expression, persistence, viral recombination or replication have led to the development of improved and safer vectors with regulatable promoters, which will be tested in this proposed project. Initial studies will address transgene expression (lacZnl or GDNF) in normal African green monkeys, determining effective titers, transduction efficiency, cellular tropism, distribution, level, and stability of transgene expression, neuropathology and host cellular responses after delivery by rEIAV or rAAV. Each of the two vectors will then be used to deliver GDNF to the nigrostriatal system of MPTP parkinsonian monkeys to test hypotheses that GDNF expression will improve function in both moderate and severely parkinsonian monkeys for periods up to 24 months. The most effective procedures will be optimized by comparing injection sites, a regulatable promoter to inactivate gene expression, and safety of all procedures including high injection titers. Measures of efficacy will include behavioral parameters, molecular assays of transgene expression using ELISA for protein, RT-PCR for mRNA and PCR for vector DNA, biochemical assays of DA and its metabolites, neuroanatomical and morphometric analyses, neuropathology, clinical chemistry, SPECT imaging, and autoradiography. These studies aim to provide the necessary data to initiate successful clinical trials in Parkinson's patients at the earliest possible time. -

Principal Investigator: ROCCA, WALTER A.
Grant Number: 5R01NS033978-09
Title: EPIDEMIOLOGY AND GENETICS OF PARKINSON'S DISEASE

Abstract: Parkinson's disease (PD) is a common and disabling condition in the expanding elderly population of the US and worldwide. Its etiology remains unknown and both genetic and environmental factors have been suspected. The long-term goal of the proposed studies based in different sampling is to clarify the etiology of PD and to identify means to prevent it. Three independent but related studies based on different sampling and measurement strategies are proposed. The hypotheses tested derive directly from our current work and preliminary findings. A case control study will include 800 cases of PD referred to the Mayo clinic from a 120 mile radius or from a 5 state region and 800 controls free pf PD and parkinsonism matched by age (+ 2 years), sex and region of residence. Controls from the general population will be identified from health care financial administration lists for cases aged 65 years or above and through random digit dialing for cases below 65 years. Exposures will be accessed through direct telephone interview, and will include tobacco, coffee, and alcohol use; markers of novelty seeking behavior; and, for women, use of estrogen replacement therapy after menopause and other reproductive and estrogen related factors. A first historical cohort study will test the association between unilateral and bilateral oophorectomy before menopause and PD in an established population based cohort. The study will include 2,533 women who underwent oophorectomy in 1950-1987 while residing in Olmsted County, MN and 2,533 women of the same age and residence who did not undergo oophorectomy. A second historical study will test the association between personality traits measured by the Minnesota Multiphasic Personality Inventory (MMPI) and PD in an established research cohort. This study will include 8,775 persons who underwent MMPI testing in 1962-1965 while residing in Minnesota. The proposed case-control study is strong because it has adequate statistical power to confirm our preliminary findings on the role of estrogen in PD and to explore the link between substance use and novelty seeking behaviors in PD. All interviews with case and controls will be direct, the proposed oophorectomy cohort study is strong because of its cohort design, its population-based sampling, its adequate statistical power, and because of the expected high rate of follow-up through both passive and active strategies. The proposed MMPI cohort study is strong because of its cohort design, its adequate statistical power and because of our extensive experience with tracing and interviewing individuals. These three studies will contribute greatly to understanding the causes and possible prevention of PD by exploring novel hypotheses and by

Principal Investigator: RON, DAVID
Grant Number: 3R21NS043628-02S1
Title: Endoplasmic Reticulum Stress and Parkinson's Disease

Abstract: Unavailable

Principal Investigator: RUOHO, ARNOLD E
Grant Number: 5R01NS033650-09
Title: Characterization of Vesicular Monoamine Transporters

Abstract: The strategy of this proposal is based on the rationale that identification of the inhibitor, substrate, proton translocation, and functionally relevant phosphorylation sites on monoamine transporters (VMAT2) will provide a basic understanding of the mechanism of action of monoamine sequestration into vesicles and the factors which regulate transporter activity. This work will be accomplished in three Specific Aims: (1) Identification of the reserpine binding site(s) on VMAT2. Novel reserpine photoaffinity labels will be synthesized and characterized, and photo-labelled peptides will be identified in order to map the reserpine binding site; (2) Identification of the substrate transport channel. This aim will involve the use of several approaches, including radioactive photo-activatable substrate analogs to covalently derivatize the substrate binding site on VMAT2; site-specific derivatization of VMAT2 at engineered cysteine residues with the cysteine-reactive reagents, methanethiosulfonate ethyl amine (MTSEA), and MTS-ethyltrimethylammonium (MTSET); and site-directed mutagenesis of potential residues lining the channel; (3) Determination of the functional role of two highly charged regions of VMAT2. This aim will involve the use of biochemical and genetic (site-directed mutagenesis) approaches to determine the role of phosphorylation of the N-terminus of VMAT2 on transporter function and the intracellular distribution/oligomeric state of the transporter. Reduced or aberrant activity of the monoamine transporter of the synaptic vesicles in dopaminergic neurons of the substantia nigra through either direct or indirect actions of toxicants (e.g., MPP+, insecticides) and genetically altered neuronally expressed proteins may play a central role in Parkinson's Disease. The regulation of uptake of monoamine neurotransmitters into storage vesicles may also play an important role in affective psychological disorders related to depression by altering levels of serotonin, norepinephrine, dopamine, or other neurotransmitters. This work will provide insight into the mechanism of action of the monoamine transporters and contribute to our understanding of how pharmacological and therapeutic strategies may be devised to treat Parkinsonism or other disorders of the nervous system. -

Principal Investigator: SALVATERRA, PAUL M
Grant Number: 5R01NS019482-21
Title: Genetic Studies of the Cholinergic Locus

Abstract: Unavailable

Principal Investigator: SARANG, SATINDER S
Grant Number: 1R43NS050920-01
Title: PESTICIDE-SYNUCLEIN INTERACTIONS AS RISK FACTORS FOR PD

Abstract: Parkinson's disease (PD) and other age-associated neurological disorders represent one of the largest unmet medical needs in developed countries. However, the discovery of improved diagnostics and therapeutics for these disorders is hampered by incomplete understanding of underlying disease mechanisms and risk factors. Oxidative stress, mitochondrial dysfunction, and protein aggregation have been implicated as major mechanisms causing dopaminergic neuronal loss in PD. Epidemiological studies have revealed an association between pesticide exposure and PD, and pesticides that cause oxidative stress and mitochondrial dysfunction, such as rotenone and paraquat, are used in cellular and animal models of PD. Furthermore, interactions between pesticides and the PD-linked gene alpha-synuclein have been postulated. Although almost 1000 pesticide active ingredients are currently marketed, these compounds have not been systematically screened for neurotoxicity in cellular or animal models of PD. The identification of pesticides that interact with alpha-synuclein to cause neurodegeneration may lead to the discovery of novel candidate risk factors and more representative disease models for PD. For this proposal, investigators at Cambria Biosciences will exploit a published moderate-to-high throughput neuronal cell-based model of PD, with the goal of identifying individual pesticides and synergistic pesticide combinations potentially involved in the pathogenesis of PD. Our established cellbased model of PD will be used to screen -approximately 350 registered pesticides to identify neurotoxic pesticides. Our specific aims include: (1a) identifying neurally-active pesticides that induce cell injury to two PD-like cell lines that stably express wild type (WT) human alpha-synuclein and mutant A53T alpha-synuclein; (1b) identifying any synergistic effects of neurotoxic pesticides in inducing cell damage in these a-synuclein-expressing neuronal cells; and (2) characterizing the activity of these neurotoxic pesticides and pesticide combinations using primary mature mesencephalic DA neurons. The identified neurotoxic pesticides will be employed in follow-on Phase II studies for the development of improved in vitro and in vivo PD models, which will ultimately be used to screen for neuroprotective compounds as part of a comprehensive drug discovery program. -

Principal Investigator: SCHLOSSMACHER,
Grant Number: 2K08NS002127-04
Title: The Roles of a-Synuclein and Parkin in Parkinson Disease

Abstract: The pathogenesis of Parkinson disease (PD) is unknown but dopamine-induced oxidative stress, proteasomal abnormalities and mitochondrial dysfunction are associated with its neurodegeneration. Rare heritable forms of PD are linked to an increasing number of gene loci. At the PARK1 locus, SNCA encodes a neuronal protein, alpha-synuclein (alpha-S), that is involved in the transition of synaptic vesicles from the reserve-resting pool to the readily releasable pool in vivo and in vitro. It is linked to sporadic PD by the formation of fibrillar inclusions that contain phosphorylated alpha-S, and to autosomal dominant PD by a likely gain-of-function effect of two infrequent point mutations. The PARK2 gene encodes parkin, an E3 ubiquitin ligase. It is mutated in < 50% of all autosomal recessive PD cases by a probable loss-of-function phenomenon. In normal human brain (but not rat brain), a pool of alpha-S undergoes O-linked glycosylation, thereby generating alpha-Sp22. This glycoprotein is a substrate for parkin's E3 ligase function in vitro and accumulates in PARK2-mutant PD brain. The central hypotheses of this application state that 1) a shared pathogenetic pathway is encoded by PD-linked genes, 2) characterization of the alpha-S glycosylation in primate brain will provide insights into the pathogenesis of PD, 3) the normal function of the Parkin E3 complex is essential for the sustained survival of catecholaminergic neurons in adult human brain, and 4) the identification of the in vivo subunits of the assembled parkin E3 complex will validate reported binding partners and reveal potentially neurotoxic substrates. To this end, I have identified two Specific Aims: Aim 1: To characterize the glycosylation of alpha-S in human control brain as well as PARK1-linked PD brain and to model its biosynthesis in a cell model, and Aim 2: To biochemically purify the subunits of the Parkin E3 ligase complex from human brain, and verify them in vitro.-

Principal Investigator: SCHWARTZ, LAWRENCE
Grant Number: 5R01NS042898-02
Title: Do Ariadne and Parkin Share Redundant Function

Abstract: Unavailable

Principal Investigator: SHEN, JIE
Grant Number: 5R01NS041779-04
Title: Studies of Parkin KO Cells and Mice as PD Models

Abstract: Parkinson's disease (PD) is an age-related neurodegenerative disorder affecting approximately 5% of people over age 65. PD is characterized pathologically by the selective degeneration of dopaminergic neurons in the substantia nigra and the formation of intraneuronal inclusions known as Lewy bodies. Recessively inherited mutations in the Parkin gene are the most common cause of inherited and early onset PD. A variety of large Parkin deletion and truncation mutations as well as missense mutations have been linked to PD in many families, strongly indicating that recessively inherited parkinsonism is caused by loss of Parkin function. The central hypothesis underlying our research is that loss-of-function mutations in the Parkin gene alter the normal physiology of dopaminergic neurons in the substantia nigra, ultimately leading to the parkinsonian phenotype. A loss-of-function pathogenic mechanism can be studied in cells and animals from which the Parkin gene has been deleted. Knockout mice are commonly used to investigate the normal function of genes. Knockout mice can also be used to study diseases caused by gene deletions in humans. Parkin knockout mice can be used to study the abnormal nigral degeneration caused by loss of Parkin function in humans. To investigate the role of Parkin in the survival of dopaminergic neurons, we propose to generate mice with targeted germ-line disruption of the Parkin locus. The Parkin knockout mice will then be analyzed for biochemical and neuropathological abnormalities associated with PD, such as degeneration of dopaminergic neurons, reductions in striatal dopamine levels, and motor behavioral deficits. In parallel, we will generate and analyze Parkin knockout cells in vitro. This will provide a powerful cellular system with which to characterize the function of Parkin and to examine the consequences of its absence, such as increased sensitivity to oxidative stress and apoptotic stimuli. Both the animal and the cellular systems could provide valuable means for identifying and testing molecules and genes with therapeutic potential. -

Principal Investigator: SHERMAN, MICHAEL Y
Grant Number: 1R01NS047705-01A1
Title: Cell Mechanisms of Abnormal Protein Aggregation

Abstract: Neuronal accumulation of various mutant or damaged proteins results in several neurodegenerative disorders, including Parkinson's disease, ALS, and polyglutamine expansion disorders. Toxic abnormal species can aggregate in cells, and there is an ongoing discussion of how protein aggregation influences neurotoxicity. It has recently become clear that in contrast to protein aggregation in a test tube, aggregation of damaged or mutant polypeptides in vivo is a complicated and tightly regulated process that involves many cellular factors. Using a yeast model of polyglutamine (polyQ) expansion disorders, the PI has carried out a number of genetic screens and found that mutations in several components of the machinery that organizes cortical actin patches (CP) dramatically reduce polyQ aggregation. Furthermore, elimination of CP by arp2 or arp3 mutations completely blocks aggregation of polyQ in cells. This proposal will test the hypothesis that cortical actin patches play a direct role in polyQ aggregation. Accordingly, investigations will be carried out to determine if polyQ-containing polypeptides aggregate at CP sites, and the role of Rnq1 prion in these interactions. The role of components of CP and factors responsible for formation of actin cables in polyQ aggregation will be evaluated. An important goal would be to establish whether CP play a general role in protein aggregation, including aggregation of distinct proteins important for neurological disorders, e.g. synphilin 1, alpha-synuclein and PABP2, and in formation of yeast prions. A critical question will be whether homologs of major components of CP play similar role in polyQ aggregation in mammalian cells. A special focus will be to evaluate a hypothesis that interactions between CP and polyQ are mediated by certain SH3-domain proteins, e.g. Sla1, Rvs167, Bem1 or Hof1. Previous work from the PI's lab showed that polyQ aggregation causes early defect in endocytosis in yeast and mammalian cells. In a separate aim we will test a hypothesis that polyQ aggregation causes inhibition of endocytosis in neurons, using a C. elegans model. It will also be established whether mutations that increase the lifespan and delay the onset of polyQ aggregation in worms also delay the onset of endocytosis defects. Exploration of fundamental mechanisms of protein aggregation that we undertake in this project will help to understand the nature of several neurological disorders. -

Principal Investigator: SILVERMAN, RICHARD B
Grant Number: 1R01NS047331-01A1
Title: Celestrols for Treatment of Neurodegenerative Diseases

Abstract: The expression of molecular chaperones has been shown to suppress protein misfolding/aggregation and cellular toxicity phenotypes in model systems associated with Huntington's Disease, Alzheimer's Disease, Parkinson's Disease, and ALS. A feature common to diseases of protein conformation is the appearance of folded intermediates that self-associate to form protein aggregates and inclusions. The molecular chaperones Hsp90 and Hsp70 sequester damaged proteins that appear in cells exposed to physiological and environmental stress. The ability of molecular chaperones to suppress the cellular toxicities associated with expression of these "toxic" proteins may be due to the intrinsic properties of chaperones to capture and suppress the appearance of folded intermediates. Therefore, we propose that the identification of small molecules that elevate the expression of genes encoding heat shock proteins and molecular chaperones should lead to the development of novel therapies beneficial to the prevention of neurodegenerative diseases. The rationale for this proposal is based on results obtained by our laboratory and others who participated recently in a screening program organized by the NINDS, Huntington Disease Society of America, Hereditary Disease Foundation, and the ALSA to identify new drugs for treating these diseases. A search was carried out for drugs that activate the heat shock response; the most effective compound identified was the natural product celastrol. Synthetic analogs of celastrol will be prepared to optimize its effectiveness as a regulator of the heat shock response and a suppressor of neurotoxicity and to determine its mechanism of action as an activator of the heat shock response. To probe the function of celastrol as a potential therapy for neurodegenerative diseases, the following Specific Aims will be addressed: (1) Synthesize analogs of celastrol that induce the human heat shock response using a heat shock promoter-reporter assay in human tissue culture cells. (2) Determine the mechanism of action of celastrol (or an analog). The working model is that celastrol activates the heat shock response by inducing heat shock transcription factor HSF1. The mechanism by which HSF1 activity is induced by celastrol will be determined. It also will be determined whether celastrol, by virtue of its ability to activate the expression of chaperones, can reduce the aggregation and neurotoxicity of the Huntington Q64 protein expressed in a human SH-SY5Y neuroblastoma cell line. (3) Studies will be carried out to identify the binding target for celastrol using molecular biological and biochemical techniques. Identified target(s) will then be cloned and characterized. Results of these studies

Principal Investigator: SIMON, DAVID K
Grant Number: 5K02NS043311-03
Title: Acquired Mitochondrial DNA Mutations in the Brain

Abstract: Mitochondrial complex I (CI) activity appears to play a key role in the pathogenesis of Parkinson?s disease (PD). Cl activity is impaired in the substantia nigra (SN) in PD, and Cl inhibitors induce parkinsonism when systemically administered in animals. Indirect evidence suggests a role for mitochondrial DNA (mtDNA) mutations. However, detection of these mutations may not be possible by standard screening techniques, particularly if they are present at low mutational burdens. We hypothesize that numerous acquired mutations, each individually present at a low mutational burden, could reach a sufficient aggregate burden to cause mitochondrial dysfunction. Such mutations are hypothesized to result from oxidative damage to mtDNA. Brain levels of 8-hydroxy-2?deoxyguanosine (OH8dG), a marker of oxidative DNA damage associated with point mutations, are 16-fold higher in mtDNA than in nuclear DNA, increase with aging, and increase further in PD. We have developed a protocol for detecting mtDNA mutations present at extremely low mutational burdens. We propose to determine the frequency of oxidative stress-induced mtDNA mutations in frontal cortex and substantia nigra in controls and in PD. Using Laser Capture Microdissection (Arcturus), we will examine the specific subpopulation of neurons susceptible in PD. Single-cell PCR will allow us to address the question of accumulation of individual acquired mutations within single neurons. We also propose to establish an in vitro system for analyzing the induction of oxidative stress-induced mutations in dividing and in post-mitotic cells by exposure to hydrogen peroxide or to agents that inhibit mtDNA repair. Collaborations have been established which will make the proposed studies possible. These studies will lay the foundation for future studies addressing the role of acquired mtDNA mutations in aging and in neurodegenerative diseases.-

Principal Investigator: SMEYNE, RICHARD J
Grant Number: 2R01NS039006-04A2
Title: Genetics of MPTP-Induced Parkinsonism

Abstract: Parkinson's disease (PD) is a debilitating neurological disorder that strikes 20 per 100,000 persons greater than 50 years of age. It is estimated that 1 million US citizens have PD, with adults over 60 having a 1 in 20 chance of getting PD. At an average per capita cost of $6000.00 year/patient, the total cost of the disease approximates $6 billion dollars, of which 85% is borne to private and government insurance agencies. Since the population of the world is getting progressively older, the number of people suffering from this disease should substantially increase within the next several decades. The cause of >90% of all PD cases is unknown. Current hypotheses on the etiology of idiopathic PD (IPD) state that there is an interaction of some as yet unknown environmental agent with a genetic predisposition to its effects. The discovery of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) has provided a useful model of Parkinsonism that appears to recapitulate the pathology of the disease seen in man. Exposure to this prototypical "environmental toxin" causes a selective loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc). In mice, the effects of MPTP are strain dependent. We have used a QTL analysis to demonstrate that the gene underlying strain differences is located on chromosome 1. Within this chromosomal region, one gene: glutathione-S-transferase pi2 functions within the detoxification pathway for exogenous agents. In this application, we propose to study the structure and function of this gene and its related family members. Four specific aims are proposed: 1) Determine if there are any differences in the sequence and expression of GSTp2 and related family members in MPTP-resistant and sensitive strains of mice. 2) Examine the effects of blockade or transfer of GSTpi on cell death following administration of MPTP in vitro and in vivo; 3) Develop the rotenone model of experimental Parkinsonism in mice and determine if GSTp2 is altered in response to rotenone; 4) Determine if there are structural or expression differences in GSTpi levels in humans with Parkinson's disease. The results of this study should lead to a better understanding of the pathogenesis of experimental and possible human Parkinson's disease. This identification of GSTp2 as a candidate gene could also lead to the identification of diagnostic measures and point to potential therapies for early intervention in this devastating illness. -

Principal Investigator: SMITH, AMANDA D
Grant Number: 1K01NS045698-01A1
Title: Endogenous neuroprotective agents in Parkinson's disease

Abstract: The present application describes the research and career plan laid out for my development into an independent, productive, and well funded investigator in the area of the neurobiology of neurodegenerative disease. The research plan that is proposed investigates the role of circulating insulin like growth factor (IGF-1) and associated proteins in protection of the nigrostriatal dopamine (DA) pathway against oxidative stress induced by 6-hydroxydopamine (6-OHDA) and the nature of this protection. The loss of DA neurons in this pathway underlies the motor dysfunctions observed in patients with Parkinson's disease (PD). Forced use of the impaired forelimb for 7 days in a unilateral 6-OHDA lesion model of Parkinson's disease, ameliorates behavioral asymmetry and restores DA content in the striatum when commenced immediately after or prior to neurotoxic insult. The mechanism by which forced use protects against 6-OHDA toxicity is unknown. Moreover, whether forced use protects the nigrostriatal pathway from degenerating, rescue cells in danger of degenerating in the absence of intervention, or promotes sprouting, is not known. Physical exercise by treadmill or running wheel has been shown to increase the brain uptake of IGF-1 from the circulation and this IGF-1 has been shown to mediate exercise-induced increases in neurogenesis and brain derived neurotrophic factor mRNA in the hippocampus. Thus, it may be surmised that forced use protection is mediated via increases in brain IGF-1 subsequent to increases in circulating IGF-1. Our preliminary data using Fluoro-jade B as a marker of degeneration suggests that forced limb use prevents the nigrostriatal pathway from degenerating. Further, a preliminary screen of altered genes after 6-OHDA and 6-OHDA +/- forced limb use, with microarray analysis suggests that IGF-1 may be involved. In the present proposal, we will: 1) Further examine the impact of forced use/disuse on the anatomical and functional state of DA neurons using behavior, biochemistry and histological analyses; 2) investigate the role of IGF-1 in forced limb use-induced protection, whether this effect can be mimicked by systemic administration of IGF-1 and whether subsequent up-regulation of other trophic factor signaling (i.e. GDNF and BDNF) is involved; and 3) examine whether the protective effects of forced limb use and IGF-1 are mediated via activation of the pro-survival phosphatidylinositol 3-kinase (PI 3K)/Akt and extracellular signal-regulated kinase (ERK) signaling cascades. The career development plan in the present proposal focuses on providing me with the technical skills needed to accomplish the Aims outlined in the present proposal. Further, it will provide the skills and

Principal Investigator: STAROPOLI, JOHN F
Grant Number: 1F31NS048668-01
Title: Parkin and Its Regulation of Neuronal Apoptosis

Abstract: Mutations in parkin underlie an autosomal recessive form of Parkinson's disease, the second most common neurodegenerative disease. To test a working model of parkin as a component of a multi-subunit, SCF-like ubiquitin ligase complex that protects dopamine neurons from apoptosis, other components of the complex, including sel-10 and cullin-1, will be dowregulated by RNA interference in murine primary neuronal cultures. Downregulation of these components will be evaluated for potentiation of dopamine neuron apoptosis and compared to the effects of downregulating parkin itself. To test the hypothesis that a candidate substrate of the parkin-associated complex, cyclin E, is a key mediator of the apoptotic cascade(s) against which wildtype parkin normally protects neurons, pharmacological inhibition of cyclin E-associated activity will be evaluated for rescue of dopamine neurons in the context of parkin, sel-10, or cullin-1 downregulation. Finally, lentivirus-mediated overexpression of parkin in the same primary culture system will be assessed for protection of dopamine neurons from neurotoxins as compared to overexpression of mutant forms of parkin, including clinically defined mutations and forms deleted in the ubiquitin homology and RING domains.-

Principal Investigator: SZETO, HAZEL H
Grant Number: 1R21NS048295-01
Title: Cell-Permeable Peptides for Mitochondrial Protection

Abstract: The application is submitted in response to the Program Announcement (PAR-02-138) requesting applications for exploratory/developmental projects in translational research. This proposal seeks to identify candidate therapeutics for neurodegenerative disorders. We have recently discovered a small lipophilic cationic peptide DAPL (Dmt-D-Arg-Phe-Lys-NH2, where Dmt = 2', 6'-dimethyltyrosine) that is cell permeable and selectively targets mitochondria. Preliminary studies with isolated mouse liver mitochondria have shown that this small peptide can protect against mitochondrial permeability transition and swelling, and reduce accumulation of reactive oxygen species. By protecting against mitochondrial dysfunction, this peptide may potentially be useful in the treatment of numerous neurodegenerative disorders. We are now seeking short-term support to further explore the pharmacology of this lead peptide analog in protecting brain mitochondria against various mitochondrial toxins, and to discover new analogs of this peptide that might lead directly to a therapy development project for a particular neurological disorder. Our specific aims are as follows: 1) To examine the ability of DAPL to protect mitochondria dysfunction caused by calcium overloading, 3-nitropropionic acid (3NPA), 1-methyl-4-phenylpyridium ion (MPP+), and t-butyl hydroperoxide (tBHP; 2) To examine the ability of DAPL to protect against cell death caused by glutamate, 3NPA, MPP +, and tBHP; 3) To carry out structure-activity relationship (SAR) studies with DAPL analogs to identify the optimal peptide analog for further preclinical development. The results from these exploratory studies will guide us to the development of preclinical animal studies for evaluating the therapeutic potential of DAPL analogs in the treatment of stroke and various neurodegenerative disorders, including Parkinson's disease, Huntington's disease and Alzheimer's disease. Potential collaborators for the animal studies have already been identified.-

Principal Investigator: Tanner, Caroline M.
Grant Number: 5R01NS040467-05
Title: TWINS AND RISK OF PD: A CLINICAL AND IMAGING STUDY

Abstract: The long-term goal of this research is to determine the relative contributions of genetic and environmental factors in the etiology of typical Parkinson's disease (PD) by comparing concordance rates in MZ and DZ twin pairs, at least one of whom has Parkinson's disease. This proposal extends our recent results in an irreplaceable cohort, the NAS/NRC World War II Veteran Twins cohort, which showed nearly identical concordance rates in monozygotic and dizygotic twin pairs with typical late onset (over age 50) Parkinson's Disease. These results strongly implicate environmental factors in the pathogenesis of Parkinson's disease, since one would expect monozygotic twins to show a much higher concordance rate than dizygotic twins if Parkinson's Disease were genetically determined. However, without follow-up we cannot be certain that unaffected cotwins would not have eventually developed the disease, thus changing the study outcome. We propose to assess the presence of both clinical parkinsonism and abnormal striatal dopamine function in twin pairs discordant for Parkinson's disease, and to compare concordance rates by zygosity. Aim 1 will determine if long-term follow-up will change Parkinson's disease concordance ratios in monozygotic and dizygotic twins. We expect to add at least 100 newly diagnosed twin pairs and to re-assess diagnosis in 140 prevalent discordant pairs. The second aim will be to compare the concordance rates in monozygotic twins and dizygotic twins, for either Parkinson's disease or abnormal striatal dopamine function as measured using dopamine transporter imaging with [123I]beta-CIT (2beta-carbomethoxy-3beta-(4-iodophenyl) and SPECT. In the third aim, we will compare concordance rates by zygosity for an abnormal rate of decline in striatal dopamine function. The mean annual decline in striatal dopamine uptake will be estimated by using [123I]beta-CIT uptake separated by, on average, two years. These studies, by virtue of utilizing both clinical and imaging measures, should determine clearly and beyond a doubt if the earlier twins study was flawed by virtue of missing pre-clinical cases of Parkinson's disease. This in turn could help set the research agenda on the cause of Parkinson's disease for years to come. -

Principal Investigator: TESTA, CLAUDIA M
Grant Number: 5K08NS044267-03
Title: Mitochondrial dysfunction in neurodegenerative disease

Abstract: Like most neurodegenerative disorders, Parkinson disease (PD) has a chronic, slowly progressive course, selective neuronal loss, and a small percentage of familial cases caused by mutations in widely expressed genes. A simplified, reproducible and relevant model system that allows study of progressive neuronal injury would permit us to examine mechanisms of chronic neurodegeneration in PD, and allow us to screen potential neuroprotective agents. Organotypic "slice" culture models offer major advantages in that they are simplified compared to in vivo models, yet unlike dissociated cell cultures they involve the use of mature neurons, remain viable in culture for months, and maintain substantial intact circuitry and neuronal-glial interactions. We propose to characterize and use such a model to specifically examine mechanisms of neuronal injury in PD. Mitochondrial dysfunction has been proposed as a factor underlying dopaminergic cell loss in PD. There is growing evidence of decreased mitochondrial function and increased oxidative stress in human PD. In a new animal model of PD, systemic infusion of the mitochondrial toxin rotenone, an organic pesticide, causes degeneration of the nigrostriatal pathway that is highly selective, even in the presence of global mitochondrial inhibition. In the current proposal we will: 1) Optimize and characterize a rotenone model of PD in chronic organotypic slice cultures. We present data from preliminary studies demonstrating the successful use of slices containing substantia nigra pars compacta dopaminergic neurons for this purpose. 2) Exploit the unique advantages of this system to investigate the mechanisms of action of mitochondrial inhibition. We will examine the role dopamine itself plays in neuronal vulnerability, and look for evidence of oxidative damage and apoptotic cell death. 3) Investigate the interaction of genetic defects with environmental stressors in PD. We will use transgenic mouse models to examine how rotenone interacts with genetic mutations that produce familial PD. We will study how underlying genetic lesions that affect oxidative stress and apoptosis pathways may predispose cells to damage from exogenous toxins. 4) Test potential neuroprotective agents in a model of chronic neurodegeneration that is highly relevant to PD. The research outlined above is part of a customized five-year plan of training and career development for the Principal Investigator. The proposal includes active mentoring by experienced scientists, access to diverse resources, and an environment uniquely suited to help the PI develop as an independent physician scientist.-

Principal Investigator: VAN DEERLIN, VIVIANNA
Grant Number: 5K08NS041408-04
Title: Tau Isoform Expression in FTDP-17

Abstract: Filamentous aggregates of hyperphosphorylated tau are the signature brain lesions of frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP 17), an inherited tauopathy with diverse, phenotypes caused by different tau gene mutations. Tau is a microtubule (MT) binding protein that promotes tubulin polymerization into MTs and stabilizes MTs. The adult human brain contains six tau isoforms, half with 3 3R isoforms) and half with 4 Cterminal MTbinding repeats (4R isoforms) generated by alternatively splicing of exon 10 (E 10). Tau gene mutations cause FTDP 17 by impairing E 10 alternative splicing or tau functions. A puzzling aspect of the FTDP 17 syndromes is that different tau mutations damage selected subtypes of neurons and glia. Our first hypothesis is that this selective vulnerability may reflect cell type specific perturbations of tau isoforms to cause varied phenotypes. To test this hypothesis, we will determine mRNA expression profiles of tau isoforms in normal brain cell subpopulations. Although the ratio of 3R to 4R tau is 1:1 in normal human brain, it has never been determined in subpopulations of neurons and glia. In, Aim 1, we will microdissect neurons and glia from paraffinembedded tissue sections of control brains, perform linear amplification on extracted RNA followed by quantitative realtime RTPCR to measure the relative levels of each tau isoform mRNA. In Aim 2, we will similarly study the same neuronal and glial cell populations in FTDP 17 brains. Correlation of these data with disease phenotypes will clarify mechanisms of FTDP1 7. Since FTDP1 7 mutations produce different phenotypes in the same kindred, a second hypothesis proposes that altered expression of a second gene, which interacts with the tau gene or protein, influences development of phenotypic manifestations of FTDP 17 in different affected family members. Aim 3 tests this hypothesis by examining the differential expression of candidate genes (i.e. those involved in splicing, RNA stability, tau function, other cellular processes) between affected and unaffected FTDP 17 brain regions and control brains using custommade cDNA macroarrays. The completion of these Aims will advance understanding of FTDP 17 and related tauopathies, and may provide new targets for diagnosis and therapeutics. -

Principal Investigator: VAN DER WALT, JOELLE
Grant Number: 1L30NS050033-01
Title: Mitochondrial dysfunction in Parkinson's disease

Abstract: Unavailable

Principal Investigator: VANCE, JEFFREY M
Grant Number: 2P50NS039764-06
Title: The Genetics of Parkinsonism

Abstract: This is a continuation application of our very successful Morris K. Udall Parkinson Disease Research Center of Excellence, seeking to identify genes that contribute to risk of developing PD. Four projects and two cores are proposed. Project I, "Candidate genes and complex interactions in PD," continues the association studies of potential susceptibility genes with PD, derived from biological candidates and the gene expression studies of Project II. Additional specific aims are gene-gene and environmental-gene interactions. Project II, "Expression Analysis and Genomic Convergence," continues and extends our expression studies of tissue obtained by our autopsy program by adding examination of the putamen and the anterior olfactory nucleus to the SN, as well as using Laser Capture Microscope to investigate specific cell types. Genes identified in project II will be tested for association in collaboration with Project I. Project III, "Mitochondrial genetics and PD," builds upon our finding of a highly significant association of mitochondrial-encoded proteins with PD, specifically the haplogroups J and K and SNP 10398, which lies in the complex I subunit ND3. Using cybrids, it looks for functional differences associated with these different mitochondrial haplogroups. It also will examine nuclear mitochondrial genes with significant differential expression in Project II for association with PD. Project IV, "Association Mapping in PD Linkage Regions," will identify PD genes in regions of linkage on chromosomes 5, 8, and 9 through a new approach, genomic "iterative" association mapping, using a new DNA pooling strategy. Once the strongest region of association is identified, haplotype-tagging will be utilized to fine map the region further. Genes lying in the region will be tested for association with PD. The projects depend heavily on our productive cores. In Core B we continue our very successful collection of PD patients and siblings, as well as our prospective autopsy program. Core C provides neuropathology support for investigation and diagnoses of autopsy material, brain banking and genotyping support for the projects. We believe that by utilizing these different but integrated approaches and resources we will be able to define the genetic contributions to PD. -

Principal Investigator: VANCE, JEFFREY M
Grant Number: 5R01NS031153-11
Title: Genomic Screen To Identify Alzheimers Disease Genes

Abstract: To identify genes influencing age at onset (AAO) in two common neurodegenerative diseases, we performed a genomic screen for AAO in families with Alzheimer disease (AD;) and Parkinson disease (PD. (Li et al, AJHG, April, 2002). Heritabilities between 40 percent-60 percent were found in both the AD and PD datasets. For PD, significant evidence for linkage to AAO was found on chromosome 1p (LOD =3.41). In addition, evidence for AAO linkage on chromosomes 6 and 10 was identified independently in both the AD and PD data sets. Subsequent unified analyses of these regions identified a single peak on chromosome 10q between D10S 1239 and D10S 1237, with a maximum LOD score of 2.62. These data suggest that a common gene affects AAO in these two common complex neurodegenerative diseases. We propose to further map and identify the genes contributing to this age-of-onset effect. We will continue to collect new AD and PD families to further map the peaks, and test candidate genes within the region for association to age of onset in these two disorders. Candidates will be prioritized using initially obvious biological candidates, then candidates that lie within the linkage peaks that are identified through Serial Analysis of Gene Expression and Microarray studies in both AD and PD (being performed in our lab in concurrent studies) and finally through fine mapping of the linkage peak for high areas of association using a DNA pooling approach and a new Single base pair- denaturing high performance liquid chromatography methodology. Candidates lying within these high association areas will be investigated further. Once identified, the genes will be investigated in collaboration with known mouse models, at present the Parkin model of Dr. Jian Feng and the APOE models of Dr. Don Schmechel of the DUMC Alzheimer Disease Research Center. Identifying age-of-onset genes may lead to treatment and delay of these late-onset disorders and a better understanding of the pathological processes they share.-

Principal Investigator: WIEDAU-PAZOS,
Grant Number: 1K08NS002240-01A2
Title: Gsk-3beta & beta-Catenin in pathophysiology of FTDP-17

Abstract: This proposal will enable the applicant to become an independent researcher in the field of inherited neurodegenerative disorders. It builds upon the candidate's background in aging research and implements a comprehensive career development plan that aims to 1) expand the breadth of research skills in the area of cell biology and genetics and enhance current research skills, 2) fill knowledge gaps in the understanding of cellular pathways in frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17), and 3) result in publications and academic leadership development. The research will be conducted at the UCLA Department of Neurology with excellent institutional support and opportunities to collaborate. The mechanisms by which mutant tau causes neurodegeneration in FTDP-17 - a group of inherited dementias linked to mutations of the microtubule-associated protein tau - are poorly understood, thereby representing a major knowledge gap in the understanding of cell death pathways in degenerative dementias. The goal of the project is to fill this knowledge gap by focusing on one candidate mechanism, by which tau misexpression may lead to neurodegeneration. We identified this mechanism in previous studies of a Drosophila model of human tau expression. The Aims focus on studies that verify and extend preliminary findings suggesting that GSK-3-beta and beta-catenin, both components of the Wnt signaling pathway, exacerbate mutant tau-induced neurodegeneration related to FTDP-17. Preliminary results suggest that beta-catenin accumulates in CNS regions vulnerable to neurodegeneration and that GSK-3-beta may be sequestered by mutant tau. The applicant will investigate the overall hypothesis that the most common tau mutation, P301L, interferes with the ability of GSK-3-beta to phosphorylate beta-catenin and that the resulting stabilization of beta-catenin triggers enhanced neuronal death. Specifically, correlations of the onset of beta-catenin accumulation and cell death will be addressed. GSK-3-beta activity and association with mutant tau that may lead to beta-catenin accumulation and neurodegeneration will be explored. The proposed biochemical and cell biological studies will initially utilize transgenic mice expressing mutant P301L tau, which model aspects of FTDP-17 clinically and pathologically. Once correlative studies have provided information regarding potential interactions of mutant tau, GSK-3-beta and beta-catenin, functional studies of these interactions are planned. -

Principal Investigator: WOLOZIN, BENJAMIN L
Grant Number: 7R01NS041786-05
Title: Ubiquitination/Receptor Signaling--Regulation by Parkin

Abstract: Mutations in the gene coding for Parkin cause a rare familial form of Parkinsonism, autosomal recessive juvenile Parkinsonism, that results in death of dopaminergic neurons in the substantia nigra. To understand how parkin causes disease, we need to understand the regulation and function of parkin. Our studies have lead us to investigate ubiquitination, which is a process that regulates protein degradation. We hypothesize that parkin regulates ubiquitination of other proteins in response to cellular contact with matrix proteins (such as collagen and laminin), and thereby controls regulation of the cytoskeleton and signal transduction by matrix proteins and their integrin receptors. Loss of parkin function could cause neurodegeneration by inhibiting matrix signaling and impairing maintenance of processes by neurons. Our preliminary data support this hypothesis by demonstrating that parkin-dependent ubiquitination is activated by cellular binding to matrix proteins. We have also identified parkin binding proteins that are associated with integrins. Conversely, cell lines that have reduced parkin expression (due to anti-sense parkin cDNA) decrease ubiquitination, retract processes upon cellular exposure to matrix proteins, and have abnormal signal transduction. The goal of this proposal is to investigate the regulation of ubiquitination by parkin (Aim 1), determine the role of parkin in regulating signaling in response to exposure of cells to matrix proteins (Aim 2) and identify common functional deficits associated with disease-related mutations in parkin. Interestingly, parkin is also linked to other forms of neurodegeneration. Parkin binds to alpha-synuclein, and in brains from donors with Parkinson's disease parkin accumulates in inclusions that contain alpha-synuclein, and shows 75% less binding of parkin to two proteins, filamin and hCDCrel2a. We intend to investigate the mechanism of parkin dysfunction by determining how parkin function is altered in Lewy body diseases, and whether oxidation or alpha-synuclein aggregation causes the dysfunction of parkin (Aim 3). The research in this proposal will provide insight into the function of parkin, determine how mutations in parkin produce disease, and provide a new window to understand the molecular pathophysiology of Parkinson's disease.-

Principal Investigator: WOOTEN, GEORGE F
Grant Number: 3P50NS039788-05S1
Title: MITOCHONDRIAL ETIOLOGIES OF PARKINSON'S DISEASE

Abstract: Unavailable

Principal Investigator: YEN, SHU-HUI C
Grant Number: 1R01NS048052-01A1
Title: Modeling Neurofibrillary Degeneration

Abstract: Progressive supranuclear palsy shares its defining pathologic signature, neurofibrillary tangles (NFT) consisting primarily of hyperphosphorylated tau, with numerous neurological diseases, including Alzheimer's disease, corticobasal degeneration, Pick's disease as well as frontotemporal dementia and Parkinsonism linked to chromosome 17. To improve our understanding of the mechanism underlying NFT formation and its functional impacts we have developed cellular models that produce tau filaments with morphological and biochemical characteristics of human tauopathies. The models consist of conditional transfectants generated from human neuroglioma [H4] and neuronal [BE(2)-M17D] cells in which transgenic production of wild-type or mutant tau is regulated via the TetOff inducible mechanism. Preliminary studies demonstrated that treatment of these cells with 4-hydroxynonneal (HNE), proteosomal or calpain inhibitors enhances the assembly of disulfide-linked tau aggregates. The results suggest that cellular insults such as oxidative stress and deregulation of proteases may play a role in the formation of NFT. We will employ this cellular model to uncover the molecular mechanism underlying tau aggregation induced by various insults. The Specific Aims of our proposal are: (1). To test if factors implicated in the etiology and pathogenesis of human tauopathies exacerbate tau aggregation in conditional transfectants, (2). To determine if the enhanced aggregation is associated with changes in tau solubility/partition, phosphorylation, degradation and oligomerization, (3). To investigate whether such exacerbated tau aggregation is associated with altered level or state of activation/activity of particular kinases, proteases and/or proteasomes, and (4). To study if progression of the exacerbated assembly of tau aggregates can be blocked through deregulating kinases/proteases. The results are likely to provide valuable information for a rational design of therapeutics to treat neurofibrillary degeneration. -

Principal Investigator: YOUNG, ANNE B
Grant Number: 2P50NS038372-06A1
Title: MGH/MIT MORRIS UDALL CENTER OF EXCELLENCE IN PD RESEARCH

Abstract: The MGH/MIT Morris Udall Center of Excellence in PD Research is taking a broad, collaborative and interactive approach to the study of Parkinson's disease. The Projects address critical questions concerning the selective vulnerability of dopamine neurons, the mechanism and consequences of Lewy body formation and alpha-synuclein aggregation, the neural systems consequences of parkinsonism and synuclein pathology, and molecular approaches for modifying this pathology. These issues will be explored using a range of systems, from yeast genetics, to mammalian cell culture, to rodent models to human postmortem material. The Center incorporates state-of-the-art technologies including high throughput yeast genetic screens to identify modifiers of synuclein aggregation and toxicity, viral vector gene transfer to study factors in mammalian cell culture and rodent models, multi-unit tetrode recordings to study striatal plasticity, fluorescence lifetime imaging to study protein-protein interactions, and laser capture microdissection and gene arrays to study transcriptional dysregulation. The Center has a Clinical and Training Core that provides care to patients with Parkinson's disease, gathers data on clinical features of the disease and response to therapy, solicits brain donations for neuropathological study, and trains outstanding clinician scientists to be future leaders in the field. The Center also has a Bioinformatics Core that serves to integrate and analyze data across the projects, and facilitate sharing of the information. The Administrative Core is charged with management of the Center and facilitating the sharing of information, ideas, and reagents among the investigators and with other components of the Udall Centers consortium. The investigators of the MGH/MIT Center are dedicated to a program of collaborative and interactive studies which will lead to better treatments for people with Parkinson's disease.-

Principal Investigator: YOUNG, ANNE B
Grant Number: 3P50NS038372-05S2
Title: MGH/MIT PARKINSONS DISEASE RESEARCH CENTER

Abstract: Unavailable

Principal Investigator: ZABETIAN, CYRUS P
Grant Number: 5K08NS044138-03
Title: DBH as a Modifying Gene in Neurodegenerative Diseases

Abstract: The applicant, Dr. Cyrus Zabetian, has spent the past three years as a postdoctoral fellow at Yale University/ VACHS. He will join the neurology faculty at the University of Washington next year where his future mentors, Drs. Thomas Bird and Gerard Schellenberg, have established a superb research program in neurogenetics. His training will include participation in laboratory meetings, seminars, structured courses, and annual scientific meetings. He will become part of a rich collaborative network of researchers with expertise in clinical and molecular neurogenetics, catecholamine biochemistry, and biostatistics. Dr. Zabetian's long-term plans are to become established as an independent laboratory investigator within five years, and remain actively involved in patient care and resident training on the neurology service. In neurodegenerative disease research, identifying genetic mechanisms underlying compensatory changes in surviving neurons promises to lead to improved strategies of diagnosis and treatment. The project proposed in this application seeks to determine if a newly discovered promoter polymorphism (C-1021T) influences regulation of the DBH gene with potential clinical consequences in Parkinson's disease (PD), and is divided into three parts. The goal of part I is to evaluate whether homozygosity for the T allele of C-1021 T, which is associated with low levels of plasma DBH enzyme, is predictive of an earlier onset and more severe symptoms of sympathetic failure in patients with PD. A group of forty subjects homozygous for either the C or T allele will be selected from a population of 400 clinic patients with PD and assessed longitudinally using indices of sympathetic function. Part II seeks to determine whether C-1021T strongly associates with DBH expression in noradrenergic tissues. Levels of DBH protein and mRNA will be compared in postmortem human adrenal medulla specimens, homozygous for either the C or T allele, using western blots and quantitative real time RT-PCR, respectively. Part III will assess whether C-1021T is directly functional. If preliminary results are favorable, two transgenic mouse lines homozygous for either the T or C allele will be created in which the proximal 2 kb of the endogenous mouse DBH promoter is replaced by homologous human sequence. Comparing plasma and tissue levels of DBH protein and catecholamines in the two lines will detect the effect of each allele on DBH expression.-